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DOI: 10.1111/dom.14101

Document Version Peer reviewed version

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Citation for published version (APA):

Ladwa, M., Bello, O., Hakim, O., Shojaee-Moradie, F., Boselli, L., Charles-Edwards, G., Stadler, M., Peacock, J., Umpleby, A., Amiel, S., Bonadonna, R. C., & Goff, L. M. (2020). Insulin clearance as the major player in the hyperinsulinaemia of black African men without diabetes. *Diabetes, obesity & metabolism, 22*(10), 1808-1817. https://doi.org/10.1111/dom.14101

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1	Insulin clearance as the major player in the hyperinsulinaemia of black African men
2	without diabetes

3

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^{Short title: Insulin clearance, hepatic fat and insulin sensitivity in black and white men}

- 26 Word count (abstract): 244
- 27 Word count (body): 3952
- **Tables & Figures**: 4 (2 tables, 2 figures).
- **29 References:** 68

- **30 ABSTRACT**
- 31

AIMS: Despite their low levels of ectopic fat accumulation, populations of African ancestry
exhibit hyperinsulinaemia and increased metabolic risk. We aimed to investigate relationships
between insulin clearance, insulin secretion, hepatic fat accumulation and insulin sensitivity in
black African (BA) and white European (WE) men.

METHODS: 23 BA and 23 WE men with normal glucose tolerance, matched for age and body
mass index, underwent a hyperglycaemic clamp to measure insulin secretion and clearance;
hyperinsulinaemic-euglycaemic clamp with stable glucose isotope infusion to measure wholebody and hepatic-specific insulin sensitivity; and magnetic resonance imaging to quantify
intrahepatic lipid (IHL).

41 RESULTS: BA men had higher glucose-stimulated peripheral insulin levels (48.1 (35.5, 65.2) x 10³ vs 29.9 (23.3, 38.4) x10³ pmol L⁻¹ x min, p=0.017) and lower endogeneous insulin 42 clearance (771.6 (227.8) vs 1381 (534.3) mL m⁻² BSA min ⁻¹, p<0.001) compared with WE 43 44 men. There were no ethnic differences in beta cell insulin secretion or beta cell responsivity to glucose, even after adjustment for prevailing insulin sensitivity. In WE men, endogenous 45 insulin clearance was correlated with whole-body insulin sensitivity (r=0.691, p=0.001) and 46 47 inversely correlated with IHL (r= -0.674, p=0.001). These associations were not found in BA 48 men.

49 CONCLUSIONS: While normally glucose tolerant BA men have similar insulin secretory 50 responses to their WE counterparts, they have markedly lower insulin clearance, which does 51 not appear to be explained by either insulin resistance or hepatic fat accumulation. Low insulin 52 clearance may be the primary mechanism of hyperinsulinaemia in populations of African 53 origin.

- 54 **KEY WORDS**: African, ethnicity, insulin clearance, insulin secretion, intrahepatic lipid
- 55

56 ABBREVIATIONS

- 57 BA: Black African
- 58 HFF: Hepatic Fat Fraction
- 59 EGP: Endogenous glucose production
- 60 FFM: Fat-free mass
- 61 iAUC: Incremental area under the curve
- 62 IHL: Intrahepatic lipid
- 63 ISR: Insulin secretion rate
- 64 MCRI: Metabolic clearance rate of insulin
- 65 NEFA: Non-esterified fatty acids
- 66 OGTT: Oral glucose tolerance test
- 67 WE: White European

68 INTRODUCTION

69

70 The multifaceted pathophysiology of type 2 diabetes (T2D) includes peripheral and hepatic 71 insulin resistance, reduced insulin clearance and beta-cell dysfunction. Insulin secreted by the pancreatic beta cell is delivered directly via the portal vein to the liver, where the majority of 72 endogenous insulin clearance occurs [1]. The predominant mechanism of hepatic insulin 73 74 clearance involves insulin binding to its receptor on the hepatocyte surface, with endocytosis and internalisation of the insulin-receptor complex and subsequent degradation [2]. Therefore, 75 76 hepatic insulin clearance is an integral part of insulin's action on the liver, with greater hepatic 77 insulin sensitivity associated with greater clearance [3]. Intrahepatic lipid (IHL) plays a key role in glucose/insulin dysregulation; while the mechanisms are not fully understood, the 78 79 accumulation of lipotoxic mediators has been found to inhibit insulin receptor activation [4]. 80 In this way, accumulation of IHL is believed to drive impairments in both insulin clearance 81 and hepatic insulin sensitivity [5-8].

82

Populations of black African (BA) ethnicity suffer a disproportionately elevated risk of T2D 83 [9, 10], yet they are relatively protected from ectopic fat deposition and exhibit lower IHL 84 relative to other ethnicities (the so-called "African paradox") [11]. Distinctive features of 85 insulin dynamics are well-documented in populations of African ethnicity [12], with an 86 87 exaggerated insulin response to glucose in BA subjects demonstrated across a spectrum of 88 glucose tolerance [13-17]. An important contributor to this phenomenon is the relatively low insulin clearance of BA populations, which has been consistently recognised [18-22]. 89 90 Reductions in insulin clearance appear to be a predictor of T2D in this ethnic group [23] and 91 may be associated with increased markers of inflammation [24].

We have previously shown that fasting hepatic insulin resistance in BA men with early T2D appears to be independent of IHL [25] and that there are ethnic differences in the relationship between ectopic fat accumulation and insulin sensitivity [26]. This has led us to hypothesise that the role of IHL in insulin clearance may differ by ethnicity. To our knowledge, this is the first study to examine the impact of BA ethnicity on relationships between insulin clearance, insulin sensitivity and hepatic fat in adult men of normal glucose tolerance.

98 METHODS

99 Study Design

100 The data were collected as part of "Soul-Deep II", a cross-sectional study of the development 101 of type 2 diabetes in men resident in South London from two ethnic groups, white European (WE) and black (West) African (BA). Metabolic assessments were performed at the Clinical 102 Research Facility, King's College Hospital, London, UK, while MRI imaging took place at 103 104 Guy's Hospital, London, UK. The study was approved by the London Bridge National Research Ethics Committee (15/LO/1121). Recruitment of subjects and data collection took 105 106 place between April 2016 and May 2018. Recruitment was carried out through advertising in 107 the local press and via South London primary care practices. All subjects provided written 108 informed consent prior to the study.

109

110 Subjects

Eligible subjects were male, aged 18-65 years, of either white European (WE) or black (West)
African (BA) ethnicity. Ethnicity was self-declared and confirmed by grandparental
birthplace. Eligible WE subjects had 4 WE grandparents with at least two of these from North
West European countries as defined by the United Nations Statistics Division (UNSD) [27].
Eligible BA subjects had 4 BA grandparents from West African countries as defined by UNSD.

Subjects were invited to a screening assessment at the Clinical Research Facility at King's
College Hospital, following a 10 hour fast, in order to undertake a screening questionnaire,
anthropometric measurements and a 2 hour, 75g oral glucose tolerance test (OGTT). Eligible
subjects were normal glucose tolerant according to World Health Organisation criteria [28].

Exclusion criteria were: a diagnosis of diabetes; treatment with oral hypoglycaemic agents, insulin, systemic steroids or beta blockers; any condition or medication considered by the investigators to have substantial impact on the study protocol or outcomes; serum creatinine of >150 μ mol/l; serum alanine transaminase level >2.5 fold above the upper limit of the reference range; sickle cell disease (trait permitted). Participants were instructed to refrain from 1) strenuous physical activity for 48-hours 2) alcohol consumption for 24-hours and 3) food and drink (other than water) for at least 10 hours prior to the study visits.

129

Hyperglycaemic clamp assessment of first- and second- phase insulin secretory function and insulin clearance

Following an overnight fast, participants were admitted to the Clinical Research Facility and 132 weighed in light clothing. A cannula was inserted into the antecubital fossa vein of the non-133 134 dominant arm for administration of the glucose infusion and a second cannula inserted 135 retrogradely into the dorsum of the contralateral hand for blood sampling. The sampling hand 136 was placed in a hand-warming unit at 55 °C in order to achieve arterialised venous blood. A 137 primed, variable rate intravenous infusion of 20% (wt/vol) dextrose was administered for 120 minutes to achieve square-wave hyperglycaemia with a plasma glucose concentration of 6.9 138 139 mmol/L above baseline, according to the protocol of DeFronzo et al [29]. Glucose, insulin, 140 and C-peptide concentrations were measured at fasting (-20, -10, 0 minutes) and at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, and 120 minutes. 141

142

143 Hyperinsulinaemic-euglycaemic clamp assessment of insulin sensitivity

The full methodology has been described [26]. In brief, a two-step hyperinsulinaemic– euglycaemic clamp with a stable glucose isotope infusion was used to assess whole-body and hepatic-specific insulin sensitivity. Participants were admitted to the Clinical Research Facility following an overnight fast and weighed in light clothing. During the basal phase, a primed

(2.0 mg/kg), continuous infusion (0.02 mg kg⁻¹ min⁻¹) of [6,6 ²H₂]-glucose (CK Gases, 148 Cambridgeshire, UK) was initiated at -120 minutes. Blood samples were taken at -30, -20, -10 149 150 and 0 minutes for basal assessments. The clamp began at 0 minutes with a primed continuous 151 insulin infusion (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) bound to albumin at a rate of 10 mU m⁻² BSA min⁻¹ for 2 hours (low dose insulin phase) for assessment of hepatic insulin 152 sensitivity. For the final 2 hours, the insulin infusion rate was re-primed and increased to 40 153 mU m⁻² BSA min⁻¹ (high dose insulin phase) for assessment of whole-body insulin sensitivity. 154 Euglycaemia (5.0 mmol/l) was achieved using variable rate 20% (wt/vol) dextrose enriched 155 156 with $[6,6 {}^{2}H_{2}]$ -glucose (8 mg/g glucose) to maintain a constant tracer-to-tracee ratio. Blood was drawn at 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230 and 240 minutes for the 157 assessment of plasma glucose concentration, isotopic enrichment and insulin concentration. 158

159

160 Magnetic Resonance Imaging assessment of IHL

161 The full imaging protocol has been reported [30]. In brief, a Dixon-based MRI sequence was 162 used on a 1.5 Tesla Siemens scanner to obtain images from the neck to the knee (excluding 163 arms). 384 contiguous, axial T1-weighted gradient-echo images with a slice thickness of 3mm 164 were acquired, from which fat and water images were produced as part of the Dixon sequence. 165 MRI data were analysed using the open source image analysis software HOROS V 1.1.7 166 (www.horosproject.org; accessed 21/10/2017) by a single, blinded analyst (OH).

In each participant, two abdominal MRI images approximately 30mm apart were selected, representing the superior and inferior sections of the liver. In each pair of water and fat MRI images, 4 circular regions of interest (ROIs) in identical positions were placed within the liver tissue. The hepatic fat fraction (HFF) was quantified in each ROI by using the formula % HFF = (F/(F+W)) *100 where F is the pixel signal intensity of the fat image and W is the pixel signal intensity of the water image. Intrahepatic lipid (IHL) was calculated as the mean of all 8 ROIs.

173 Biochemical analyses

Plasma glucose concentrations were determined at the bedside using an automated glucose
analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Ohio, USA). Plasma
insulin concentrations were determined by immunoassay using chemiluminescent technology
(ADVIA Centaur System, Siemens Healthcare Ltd. Camberly, UK). Plasma C-peptide
concentrations were determined by radioimmunoassay (Millipore Ltd, Hertfordshire, UK).
Plasma glucose isotope enrichments were measured by gas chromatography-mass
spectrometry on an Agilent GCMS 5975C MSD (Agilent Technologies, Wokingham, UK).

181

182 Calculations

183 Whole-body insulin sensitivity was quantified using the M value (mg/kg FFM min⁻¹) measured 184 during the final 30 min of the high-dose insulin phase of the clamp, calculated as total glucose 185 disposal corrected for deviations in plasma glucose concentration [26]. Whole-body insulin 186 sensitivity was also expressed as M/I, the M value corrected for the steady state insulin 187 concentration during the last 30 minutes of the clamp (mg kg ⁻¹ FFM min⁻¹)/ (pmol L⁻¹).

188

Steele's non-steady-state equations, modified for stable isotopes, were used to determine total glucose rate of appearance, Ra (µmol kg ⁻¹ FFM min⁻¹) [31]. Endogenous glucose production (EGP) was calculated by subtracting exogenous glucose infusion rate from total glucose Ra. Hepatic insulin sensitivity was expressed as the percentage suppression of EGP from basal to the final 30 minutes of the clamp (% suppression of EGP).

194

195 The clearance rate of the exogenously administered insulin infusion during the

196 hyperinsulinaemic-euglycaemic clamp (metabolic clearance rate of insulin, or MCRI) was

calculated as the insulin infusion rate divided by the insulin concentration during the steady
state period in the final 30 minutes of the clamp (mL m⁻² BSA min ⁻¹).

199

The incremental areas under the curve (iAUC) were calculated using the trapezoid rule for Cpeptide, insulin and glucose. Classical indices of first- and second-phase insulin secretion during the hyperglycaemic clamp were determined by calculating the iAUC for C-peptide for 0 to 10 minutes and 10 to 120 minutes respectively.

204

205 Parameters of beta cell function were obtained by modelling the glucose and C-peptide curves 206 during the hyperglycaemic clamp using published methods [32, 33]. Model assessments were carried out using SAAM-II 1.2 software (SAAM Institute, Seattle, Washington). The main 207 208 outputs of the model are: pre-hepatic endogenous insulin secretion (expressed as area under 209 the curve of insulin secretion rate over 120 minutes, AUC_{ISR}); beta cell glucose sensitivity of first-phase secretion (σ 1), expressed as the amount of insulin secreted in response to a rate of 210 211 increase in glucose of 1 mmol/L between time 0 and 1 minute of the study, in (pmol m⁻² BSA)/(mmol L⁻¹ min⁻¹), beta cell glucose sensitivity of second-phase secretion (σ 2), expressed 212 213 as the steady-state insulin secretion rate in response to a step increase in glucose of 1 mmol/L above baseline, in (pmol min⁻¹ m^2)/(mmol L⁻¹). 214

215

During the hyperglycaemic clamp, average (endogenous) insulin clearance was calculatedaccording to the following formula [33]:

$$Clearance_{Ins} = \frac{AUC_{ISR}}{AUC_{I} + (I_{Final} - I_{Basal}) \cdot MRT_{Ins}}$$

218

where AUC_{ISR} is the area under the curve of insulin secretion rate, AUC_{I} is the area under the curve of insulin concentration, I_{Final} is insulin concentration at the end of the study, I_{Basal} is

- insulin concentration at the beginning of the study, and MRT_{Ins} is the mean residence time of insulin, which was assumed to be 18 minutes as reported in Navalesi *et al* [34].
- 223

224 Statistical analysis

225 Log-transformation was used on skewed variables that showed a significant deviation from normality to achieve a normal distribution prior to the use of parametric tests. Data are 226 227 expressed as means (SD) for non-transformed data and geometric mean (95% confidence intervals) for log-transformed data. Significance of differences in variables between the two 228 229 ethnic groups were made using independent sample Student's t test. The strength of associations between variables of interest was assessed using Pearson's correlation. The ethnic 230 differences in relationships between endogenous insulin clearance and whole-body insulin 231 232 sensitivity, hepatic insulin sensitivity, intrahepatic lipid and MCRI, were examined by fitting a regression model between the pairs of variables with an interaction term for ethnicity. Prior 233 234 to running the regression models, collinearity diagnostics were performed for the whole cohort 235 for insulin clearance with ethnicity, hepatic fat (IHL), insulin sensitivity (M value) and insulin secretion (AUC_ISR). The VIFs for these factors were used to exclude multicollinearity. An 236 237 ANCOVA was used, with insulin secretion (AUC_{ISR}), intrahepatic lipid (IHL), hepatic insulin 238 sensitivity (% suppression of EGP) and whole-body insulin sensitivity (M value) as co-variates, to investigate ethnic differences in average endogenous insulin clearance. An ANCOVA was 239 240 used with whole-body insulin sensitivity (M value) as a co-variate, to investigate ethnic 241 differences in endogenous insulin secretion. Missing data were excluded pairwise for all analyses; in the case of the correlation analysis between IHL and insulin clearance, this led to 242 skewing of the IHL data which was therefore log-transformed for this analysis. All analyses 243 were conducted with SPSS version 25.0 and p values< 0.05 were considered statistically 244 significant. 245

246 **RESULTS**

247

248 Participant characteristics

The characteristics of the 23 BA and 23 WE men are presented in Table 1. The two ethnic groups were well-matched for age, weight and BMI and showed no difference in HbA_{1c}, blood pressure or fasting glucose (Table 1). The BA men had significantly lower fasting triglyceride levels (Table 1).

253

254 Beta cell insulin secretory function

There were no ethnic differences in fasting C-peptide or fasting insulin (Table 2). By design, there was no difference in "clamped" glucose during the hyperglycaemic clamp (BA= 12.1 (0.65) vs WE = 12.0 (0.63) mmol/L, p= 0.635).

258

Mean peripheral insulin levels were approximately 1.5-fold higher during both first and second phase of the hyperglycaemic clamp in BA compared with WE men (Figure 1a), while there were no ethnic differences in C-peptide response (Table 2, Figure 1b), endogenous beta cell insulin secretion (AUC_{ISR}) (Table 2) or sensitivity of the beta cell to glucose during first or second phase insulin secretion, σ^1 and σ^2 (Table 2). This remained the case after measures of beta cell insulin secretion were adjusted for whole-body insulin sensitivity (p = 0.512).

265

266 Intrahepatic lipid and insulin sensitivity

Data on IHL, whole body and hepatic insulin sensitivity, as previously reported by our group
[26, 30] showed IHL was lower in BA men, while there were no ethnic differences in hepatic
insulin sensitivity or whole-body insulin sensitivity by either M value or M/I (included in Table
270 2 for reference).

271

273

276

272 **Insulin clearance**

274 Average endogenous insulin clearance was almost 50% lower in BA compared with WE men 275 during the hyperglycaemic clamp (Table 2). The ethnic difference remained significant after adjusting for whole-body insulin sensitivity (M value), endogenous insulin secretion (AUC_{ISR}),

intrahepatic lipid (IHL) and hepatic insulin sensitivity (% suppression of EGP) (p < 0.001). 277

278 Clearance of exogenous insulin as determined by MCRI was also lower in BA compared with 279 WE men (Table 2).

280

Relationships between endogenous insulin clearance and intrahepatic lipid, insulin 281 sensitivity and insulin secretion 282

283

In WE men, endogenous insulin clearance was correlated with whole-body (Figure 2a & 2b) 284 and hepatic insulin sensitivity (Figure 2c) and with MCRI (Figure 2d), while it was inversely 285 286 correlated with IHL (Figure 2e). These relationships were not found in the BA men (figure 2 287 a-e).

288

289 In multiple regression analysis, an ethnicity interaction was found in the relationship between 290 endogenous insulin clearance and whole-body insulin sensitivity (Figure 2a; p-interaction = 291 0.022). An ethnicity interaction was also found in the relationship between the measurements 292 of endogenous insulin clearance and MCRI (Figure 2d; $p_{-interaction} = 0.021$). A trend was found for an ethnicity interaction between endogenous insulin clearance and hepatic insulin 293 294 sensitivity (Figure 2c; *p*-interaction=0.057). No ethnicity interaction was found between insulin 295 clearance and IHL.

297 **DISCUSSION**

298 This study comprises a comprehensive investigation of insulin clearance and its relationships 299 with hepatic fat and insulin sensitivity in white European and black African men with normal 300 glucose tolerance. To our knowledge, it is the first to demonstrate that the markedly low insulin clearance of an African origin population may occur in the absence of insulin resistance. While 301 302 the classical paradigm suggests that increased insulin resistance drives the excess diabetes risk 303 in BA populations, these findings contribute to a newly emerging (and as yet, controversial) 304 paradigm, which proposes that impairments in insulin clearance are the primary aetiological 305 mechanism of glucose intolerance in this ethnic group [35, 36].

306

307 In this study, the response to intravenous glucose in the BA men was characterised by a 308 pronounced hyperinsulinaemia compared with that of the WE men (Figure 1). While this is 309 well-documented in the literature, it has previously been described as a compensatory response by the beta cell to increased insulin resistance and/or a consequence of "upregulated" beta cell 310 311 function [14, 18, 37-39]. By contrast, in our study population we found that there were no ethnic differences in total beta cell insulin secretion, corroborating the findings of the Federal 312 313 Women's Study [22] and developing an evidence base which disputes the argument that people 314 of African ancestry typically exhibit insulin hypersecretion.

315

We acknowledge that much of the literature, including the Federal Women's study [22], report greater first phase beta cell responsivity in African-ancestry groups [38] whereas we found no ethnic differences in either first or second phase beta cell responsivity to glucose. Our results may differ as this is one of the few studies to comprise exclusively of adult men, rather than children/adolescents [18, 19, 37, 38, 40] or all-female cohorts [39, 41-45]. Two other adult non-diabetic all-male studies have examined ethnic differences in insulin responses [46, 47]; neither found increased insulin secretion in the African-ancestry subjects compared with white
subjects, consistent with our findings. It is worth considering that the paucity of male
participants in this area of the literature may have led to an overestimation of ethnic differences
in insulin secretory response [48].

326

327 We found that the peripheral hyperinsulinaemia of our BA population was due to the ethnic 328 difference in insulin clearance; furthermore, this difference persisted after adjustment for insulin sensitivity, hepatic fat content and insulin secretion rate. Whilst insulin clearance was 329 330 associated with IHL and whole body and hepatic insulin sensitivity in WE men, these associations were absent in the BA men. Insulin clearance is a highly variable process which 331 operates under the influence of multiple physiological factors. The predominant cellular 332 333 mechanism is receptor-mediated uptake and therefore a correlation is typically seen between 334 insulin sensitivity and insulin clearance [49, 50]. Our data in the WE men are in keeping with these expected relationships. In the BA men, where such relationships are not observed, we 335 336 may postulate that such mechanisms operate differently between the ethnic groups, or with 337 different dose-responses.

338

This leads us to propose that endogenous insulin clearance in BA may be determined by 339 additional factors which are independent of insulin sensitivity, e.g. in the pathways involved in 340 341 post-receptor insulin metabolism. This assertion is supported by recent evidence from a study investigating ethnic differences in the expression and activity of hepatic insulin-degrading 342 343 enzyme (IDE) and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1) between African-Americans and non-Hispanic White Americans, which reported lower IDE 344 activity in African-Americans [51]. While reduced insulin clearance is widely regarded as an 345 early response to insulin resistance [52, 53], it is also possible that changes in insulin clearance 346

are not only a compensatory mechanism but also a primary determinant of peripheral insulinlevels [36, 54] in BA populations.

349

350 Low insulin clearance has been demonstrated in African American women [22], in pre-pubertal African-American children [18, 21, 55], in indigenous adult Ghanaians [56] and, with this 351 study, in black West African men living in the UK. The finding of this distinctive physiological 352 353 characteristic in diverse populations of African ancestry is suggestive of a genetic rather than environmentally mediated mechanism. The strong heritability of insulin clearance has been 354 355 demonstrated in a Hispanic population [57] and this may also be the case in other ethnic groups. 356 Molecular mechanisms that warrant further exploration include potential ethnic differences in inflammatory activity [24], the expression and activity of insulin-degrading enzyme [51], the 357 358 liver-adiponectin pathway [58], or in liver CEACAM-1 expression/activity [2].

359

We note that clearance of exogenously administered insulin (as determined by the MCRI) is also lower in BA compared to WE, although the difference is not as marked as for endogenous insulin clearance. This may be expected, because while only endogenously secreted insulin undergoes first pass hepatic metabolism, exogenously administered insulin also undergoes both hepatic and extra-hepatic clearance (with around 60% of peripherally administered insulin thought to be cleared by the liver [59]).

366

367 Importantly, while MCRI is closely correlated with endogenous insulin clearance in WE, this 368 is not the case in BA and ethnicity has a significant impact on the relationship between the two 369 measures. We note that models have shown that while hepatic insulin clearance is lower in 370 black ethnic groups compared with whites, extra-hepatic clearance is similar [20, 21] and that 371 hepatic and extra-hepatic insulin clearance are differentially regulated [60]. As hepatic insulin

372 clearance contributes in greater proportion to endogenous compared with exogenous insulin
373 clearance, this may explain why the MCRI does not reflect endogenous insulin clearance in the
374 black African men. These finding have implications for the use of MCRI as a measure of
375 insulin clearance in black ethnic groups.

376

The strengths of this study include the well-matched ethnic groups and the use of rigorous, gold-standard methods of metabolic analysis. Unlike some previous ethnic comparison studies [48], the subject groups were tightly characterised; of single sex with metabolic status confirmed by OGTT.

381

In terms of limitations, the study is cross-sectional and is only able to recognise the presence 382 383 or absence of associations, between hepatic fat, insulin sensitivity and insulin clearance. Only longitudinal measures would be able to determine the true dynamics of these mechanisms and 384 385 determine causality. The measure of average endogenous insulin clearance does not enable 386 differentiation between hepatic and extra-hepatic insulin clearance, although it has been shown that approximately 80% of endogenous insulin is cleared by the liver [1]. Furthermore, the 387 388 influence of gut-modulated insulin secretion and clearance was not assessed. There is evidence 389 that the incretin hormones reduce post-prandial insulin clearance [61-63] and ethnic differences in incretin hormones have been recognised [33, 64, 65], albeit the data are inconsistent. 390 391 However, it is not clear whether incretins contribute to ethnic differences in insulin clearance, 392 which would be an important line of enquiry for future investigations. We also acknowledge 393 that the failure to find an association between IHL and insulin clearance in the BA men does 394 not mean that an association does not exist. Our sample size is comparable with other studies in the literature, but we may not be powered to reliably detect such associations. While the 395 narrow range in IHL among our black participants also may have hindered our ability to detect 396

a linear correlation between IHL and insulin clearance, the range in IHL that we observed is reflective of IHL in black populations, as reported in a large epidemiological study [66]. In data we have previously reported from an obese diabetic population [25], we did find a trend to an inverse relationship between insulin clearance and intrahepatic lipid in the BA participants, suggesting that there may be a threshold mechanism at play and that IHL may not have a significant role in the determination of insulin clearance until higher levels of accumulation have occurred.

404

The study comprises male subjects only and therefore may not be generalisable to women;
however, as the majority of the work in this area has been carried out in African ancestry
females [48], this cohort offers a valuable addition to the field.

408

409 In conclusion, this study demonstrates low insulin clearance in black African men despite lower 410 hepatic fat and similar whole-body and hepatic insulin sensitivity to their white European 411 counterparts. It is increasingly recognised that type 2 diabetes is a heterogenous disease, where different aetiological components may have an impact on progression rates and choice of 412 413 therapeutic strategy [67]. Ethnic disparities in treatment response have already been 414 recognised [68] and may be explained, at least in part, by inherent physiological variations. In 415 healthy black African men, the lack of association of endogenous insulin clearance with either 416 intrahepatic lipid or insulin sensitivity supports the hypothesis that low insulin clearance is a primary phenomenon in this ethnic group. Such a phenomenon warrants further exploration, 417 418 as it may offer novel therapeutic targets for the treatment and prevention of diabetes. Both the determinants of low insulin clearance and its role in the high risk of metabolic dysfunction in 419 420 African ethnic populations remain to be elucidated.

Acknowledgements

The authors would like to thank: A. Pernet, B. Wilson and M Henderson-Wilson (Diabetes Research Group, King's College Hospital, London, UK) for assisting with the metabolic assessments; T. Dew (ViaPath, King's College Hospital) for assistance with sample processing and laboratory analysis; L. Coppin and N. Jackson (University of Surrey, Guildford, UK) for assistance with analysis of the glucose enrichments; E. Giemsa (Clinical Research Facility, King's College Hospital) for accommodating the participant visits; the staff of the Clinical Research Facility at King's College Hospital for help in performing the studies; and the study participants for their time and commitment.

JLP 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

Statement of Data Availability

Data are available from the authors (LMG) on request.

Funding

This work was funded by a Diabetes UK project grant (14/0004967)

Contribution statement

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

LMG 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

Conflict of interest statement

SAA has served on advisory boards for NovoNordisk, Medtronic and Roche. The other authors declare no conflict of interest.

Legends

Table 1: Clinical characteristics of study participants.

Table 2: Metabolic parameters of insulin secretion and insulin clearance

Figure 1

a: Plasma insulin response by ethnic group during the hyperglycaemic clamp.

b: Plasma C-peptide response by ethnic group during the hyperglycaemic clamp.

Figure 2

a: Associations between endogenous insulin clearance and whole-body insulin sensitivity (M value).

b: Associations between endogenous insulin clearance and whole-body insulin sensitivity (M/I).

c: Associations between endogenous insulin clearance and hepatic insulin sensitivity (% suppression EGP)

d: Associations between average endogenous insulin clearance and MCRI

e. Associations between endogenous insulin clearance and intrahepatic lipid (IHL)

422	Table 1: Clinical characteristics of study participants.
423	

	BA (n=23)	WE (n=23)	p value
Age (years)	30.7 (12.0)	35.9 (13.9)	0.18
Weight (kg)	84.1 (14.6)	86.5 (16.5)	0.60
$BMI (kg m^{-2})$	26.7 (3.6)	26.5 (4.6)	0.86
Waist circumference (cm)	87.5 (9.3)	93.8 (14.6)	0.09
Fasting glucose (mmol L ⁻¹)	5.25 (0.4)	5.20 (0.4)	0.51
HbA _{1c} IFCC (mmol/mol)	37.0 (5.3)	35.9 (2.9)	0.37
HbA _{1c} DCCT (%)	5.54 (0.48)	5.44 (0.24)	0.38
Systolic blood pressure (mmHg)	123.1 (12.3)	121.9 (9.1)	0.70
Diastolic blood pressure (mmHg)	70.7 (11.5)	71.1 (8.2)	0.88
LDL-cholesterol (mmol L ⁻¹)	2.66 (0.87)	2.99 (0.82)	0.19
HDL -cholesterol (mmol L^{-1})	1.30 (0.42)	1.27 (0.31)	0.75
Total cholesterol (mmol L^{-1})	4.27 (1.06)	4.76 (1.05)	0.13
Triglycerides (mmol L ⁻¹)	0.68 (0.25)	1.10 (0.56)	0.003

Data presented as mean (SD). Differences between the two ethnic groups determined using independent sample

Student's t test. Fasting glucose values taken at screening visit. Abbreviations: BA black African; DCCT

Diabetes Control and Complications Trial; HbA_{1c} glycated haemoglobin; HDL high density lipoprotein; IFCC International Federation of Clinical Chemistry; LDL low density lipoprotein; WE white European.

	BA (n=23)	WE (n=23)	Mean difference or ratio of geometric mean (95% CI)	p value
Fasting plasma glucose (mmol L ⁻¹)	5.26 (0.35)	5.19 (0.32)	-0.07 (-0.28, 0.13)	0.460
Fasting plasma C-peptide $(nmol L^{-1})^{\dagger}$	0.54 (0.47, 0.62)	0.61 (0.50, 0.76)	1.14 (0.89, 1.47)	0.281
Fasting plasma insulin $(pmol L^{-1})^{\dagger}$	46.2 (38.6, 55.3)	39.4 (30.2, 51.6)	0.85 (0.62, 1.17)	0.314
iAUC _{c-pep} 0-10 mins [†] (nmol L ⁻¹ x min)	8.45 (6.17, 11.6)	6.88 (5.83, 8.3)	0.81 (0.58, 1.15)	0.240
iAUC _{c-pep} 10-120 mins (nmol L ⁻¹ x min)	242.1 (109.9)	213.2 (60.4)	-28.9 (-82.0, 24.3)	0.277
iAUC _{ins} 0-10 mins [†] (pmol L^{-1} x min)	2.46 (1.85, 3.28) x10 ³	1.75 (1.5, 2.1) x10 ³	0.71 (0.51, 0.98)	0.040
iAUC _{ins} 10-120 mins † (pmol L ⁻¹ x min)	48.1 (35.5, 65.2) x10 ³	29.9 (23.3, 38.4) x10 ³	0.62 (0.42, 0.91)	0.017
AUC _{ISR} over 120 mins (pmol m ⁻² BSA x min)	58.9 (24.0) x10 ³	54.4 (16.9) x10 ³	-4.50 (-17.1, 8.14)	0.477
$\sigma^{1 \dagger}$ (pmol m ⁻² BSA)/ (mmol L ⁻¹	692.9 (505.9, 948.9)	550.5 (464.9, 651.8)	0.80 (0.56, 1.13)	0.190
$\frac{\min^{-1}}{\sigma^2}$ (pmol min ⁻¹ m ² BSA)/	49.8 (20.1)	47.1 (18.8)	-2.67 (-14.4, 9.07)	0.649
Average (endogenous) insulin clearance	771.6 (227.8)	1381 (534.3)	609.5 (349.5, 869.6)	< 0.001
$(mL m^{-2} BSA min^{-1})$ MCRI $(mL m^{-2} BSA min^{-1})$	482.8 (70.6)	530.7 (78.7)	47.9 (1.6, 94.3)	0.043
Basal EGP	4.69 (2.39)	4.04 (2.29)	-0.65 (-2.13,0.83)	0.377
(μmol kg ⁺ FFM min ⁺) % suppression EGP [‡]	65.7 (16.1)	69.8 (17.7)	4.15 (-6.5, 14.8)	0.437
% IHL [§]	3.78 (1.13)	6.08 (5.04)	2.29 (0.06, 4.52)	0.044
M value [‡]	9.65 (2.32)	9.51 (3.86)	-0.14 (-2.08, 1.81)	0.89
mg kg ⁻¹ FFM min ⁻¹)/ (mg kg ⁻¹ FFM min ⁻¹)/	0.0171 (0.0059)	0.0189 (0.0094)	0.00184 (-0.0030, 0.0066)	0.44

 $(pmol L^{-1})$

Data presented as mean (SD) or geometric mean (95% CI) for log transformed data (†). Differences between the two ethnic groups were determined using independent samples t-tests. Fating plasma glucose levels from hyperglycaemic clamp visit. ‡ Previously reported data [26] § Previously reported data [30]

Abbreviations: BA, black African; BSA: Body surface area; EGP, Endogenous glucose production; FFM, Fat free mass. IHL, Intrahepatic lipid; WE, white European; iAUC, incremental area under the curve; AUC(ISR), area under the curve of insulin secretion rate over 120 minutes; MCRI, metabolic clearance rate of insulin.

Figure 1a: Plasma insulin response by ethnic group during the hyperglycaemic clamp. BA = black African, WE = white European. Data shown are mean and SD.



Figure 1b: Plasma C-peptide response by ethnic group during the hyperglycaemic clamp. BA = black African, WE = white European. Data shown are mean and SD.



Figure 2a: Associations between average insulin clearance and whole-body insulin sensitivity (M value) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: BA: r = 0.051, p = 0.837; WE: r = 0.691, p = 0.001. Interaction by ethnicity was assessed using linear multiple regression. FFM, fat free mass.



Figure 2b: Associations between average insulin clearance and whole-body insulin sensitivity (M/I) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: BA: r= 0.179, p=0.464; WE: r=0.697, p<0.001. Interaction by ethnicity was assessed using linear multiple regression.



Figure 2c: Associations between average insulin clearance and hepatic insulin sensitivity (% suppression EGP) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: BA: r = -0.169, p = 0.488; WE: r = 0.417, p = 0.068. Interaction by ethnicity was assessed using linear multiple regression.



 $P_{\text{interaction}} = 0.057$

Figure 2d: Associations between average endogenous insulin clearance (measured during hyperglycaemic clamp) and metabolic clearance rate of (exogenous) insulin (MCRI, measured during hyperinsulinaemic-euglycaemic clamp) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: r=0.298, p=0.215; WE: r=0.661, p=0.001. Interaction by ethnicity was assessed using linear multiple regression.



Figure 2e: Associations between average insulin clearance and intrahepatic lipid (IHL) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: BA: r = -0.134, p = 0.584; WE: r = -0.674, p = 0.001. Interaction by ethnicity was assessed using linear multiple regression.



 $P_{\text{interaction}} = 0.837$

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