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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**

4 Oluwatoyosi Bello[1], Meera Ladwa[1], Olah Hakim[1], Chinmay Marathe[1], Fariba Shojaee-

5 Moradie[2], Geoff Charles-Edwards[3,4], Janet L. Peacock[5], A. Margot Umpleby[2], Stephanie A.

6 Amiel[1] and Louise M. Goff[1]

7

8 1. Department of Diabetes, School of Life Course Sciences, Faculty of Life Sciences &

9 Medicine, King's College London, London, UK

10 2. Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK

11 3. Medical Physics, Guy's & St Thomas' NHS Foundation Trust, London, UK

12 4. School of Biomedical Engineering & Imaging Sciences, King's College London, London,

13 UK

14 5. School of Population Health and Environmental Sciences, King's College London, London,

15 UK

16

17 **Corresponding author:**
18 Louise M. Goff, Department of Diabetes, School of Life Course Sciences, Faculty of Life
19 Sciences & Medicine, King's College London, Franklin-Wilkins Building, Waterloo Campus,
20 London, SE1 9NH, UK
21 Email - louise.goff@kcl.ac.uk
22 Phone - 0207 848 4273
23 Louise M. Goff ORCID ID: 0000-0001-9633-8759
24 Oluwatoyosi Bello ORCID ID: 0000-0003-3850-0425

25

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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

ABSTRACT

Objectives: In men of black west African (BAM) and white European (WEM) ethnicity, we aimed to 1) compare adipose tissue, peripheral and hepatic insulin sensitivity, and 2) investigate associations between ectopic fat and insulin sensitivity by ethnicity.

Design and Methods: In overweight BAM (n=21) and WEM (n=23) with normal glucose tolerance, we performed a two-step hyperinsulinaemic–euglycaemic clamp with infusion of [6,6 $^{2}H_{2}$]-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).

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

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

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^2) (3) and at a lower waist circumference (4) compared to white groups.

Insulin resistance for carbohydrate metabolism is a well-established early defect in the pathogenesis of T2D (5). Resistance to the antilipolytic effect of insulin in adipose tissue has also been identified as an early defect, occurring prior to the onset of hyperglycaemia (6). Adipose tissue insulin resistance results in increased fatty acid release, with deposition in non-adipose tissue sites as ectopic fat (7, 8). This is known to trigger and exacerbate insulin resistance (9). Several theories have been proposed to explain the development of ectopic fat and insulin resistance. The "spillover theory" proposes that multiple dysfunctions of 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 lipolytic VAT leads to the release of fatty acids into the portal and peripheral circulations. The "portal theory" proposes that delivery of fatty acids from VAT to the liver, via the portal circulation, results in accumulation of intrahepatic lipid (IHL), which subsequently leads to the development of hepatic insulin resistance (12-14). Fatty acids entering the peripheral circulation are understood to lead to fat deposition within skeletal muscle cells (termed intramyocellular lipids, IMCL) (13, 15). Whilst there is compelling evidence linking IMCL with peripheral insulin resistance (16, 17), there is debate in this field due to observations that athletes, who are highly insulin sensitive, present with relatively high IMCL levels (18).

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
95 pronounced insulin resistance (24), creating a paradox. Studies using more sensitive measures
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,
127 with a BMI between 20-40 kg/m^2 were eligible to take part; the aim of recruitment was to
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.

136 Prior to each visit, participants were required not to eat after their usual carbohydrate-
137 containing evening meal (no less than 10 hours prior to study), refrain from strenuous physical
138 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
142 the clinical research facility in King's College Hospital, participants were advised to empty
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-$^{2}H_{2}$]-glucose and [$^{2}H_{5}$]-glycerol tracers (CK Gases,
151 Cambridgeshire, UK). To begin the basal phase, a primed (2.0 mg/kg), continuous infusion
152 (0.02 mg/kg^{-1} min^{-1}) of [6,6-$^{2}H_{2}$]-glucose and a primed (0.12 mg/kg), continuous infusion
153 (0.0067 mg/kg^{-1} min^{-1}) of [$^{2}H_{5}$]-glycerol were initiated at -120 minutes (36). Blood samples
154 were taken at -30, -20, -10 and 0 minutes for basal assessments. The clamp began at 0 minutes
155 with a primed continuous insulin infusion at a rate of 10 mU m^{-2} BSA min^{-1} (low dose insulin
156 phase) for 2 hours for assessment of adipose tissue and hepatic insulin sensitivity. For the final
157 2 hours, the [$^{2}H_{5}$]-glycerol isotope infusion was terminated, the insulin infusion rate was re-
158 primed and increased to 40 mU m^{-2} BSA min^{-1} (the high dose insulin phase) for assessment of
159 whole body and peripheral (skeletal muscle) insulin sensitivity (37, 38). Euglycaemia (5
160 mmol/l) was achieved using 20% glucose enriched with [6,6-$^{2}H_{2}$]-glucose (8 mg/g glucose
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 glucose
166 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 (^{1}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
180 muscle of the right leg was derived from a ^{1}H-MRS scan on a 1.5T Siemens system with an
181 extremity RF coil to obtain muscle images. From these images two localised proton spectra
182 were obtained, a water-suppressed lipid spectra and a lipid-suppressed water spectra. The Java-
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**

186 Plasma glucose and glycerol isotope enrichments were measured by gas chromatography-mass
187 spectrometry on an Agilent GCMS 5975C MSD (Agilent Technologies, Wokingham, UK)
188 using selected ion monitoring to determine the tracer-to-tracee ratio. The isotopic enrichment
189 of glucose was determined as the penta-O-trimethylsilyl-D-glucose-O-methoxime derivative

190 (41). The isotopic enrichment of plasma glycerol was determined as the tert-butyl trimethylsilyl
191 (tBDMS) glycerol derivative (42). Plasma insulin concentration was measured by
192 immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens
193 Healthcare, Camberley, UK).

194 **Calculations**

195 Whole body insulin sensitivity was quantified using the M value (mg/kg FFM min^{-1}) 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^{-1}) / (pmol/l) as another assessment of whole body insulin sensitivity (37).
200 Steele's non-steady-state equations modified for stable isotopes (43) were used to determine
201 peripheral glucose utilisation (glucose rate of disappearance, Rd (μmol /kg FFM min^{-1})),
202 endogenous glucose production (glucose rate of appearance, Ra (μmol /kg FFM min^{-1})) and
203 whole body lipolysis (glycerol Ra (μmol /kg FFM min^{-1})) at basal and during the different
204 phases of the clamp. Glucose kinetic calculations were modified to incorporate the [6,6-$^{2}H_{2}$]-
205 glucose isotope enriched 20% glucose (44). Optical segment analysis was used to smooth the
206 glucose and glycerol enrichment concentrations over the clamp time course (45).
207 Peripheral insulin sensitivity was determined as the percentage increase in the rate of glucose
208 disappearance from basal to the final 30 minutes of the high dose insulin phase. The peripheral
209 insulin sensitivity index (PISI) was also calculated as the glucose Rd (μmol /kg FFM min^{-1}) /
210 mean plasma insulin concentration (pmol/l) during the final 30 minutes of the high dose insulin
211 phase (46). Peripheral insulin sensitivity is predominantly driven by skeletal muscle glucose
212 uptake but also captures adipose tissue glucose uptake hence, we have used PISI as an
213 assessment of skeletal muscle insulin sensitivity.

214 Endogenous glucose production (glucose Ra) was calculated by subtracting the exogenous
215 glucose infusion rate from total glucose Ra. Hepatic insulin sensitivity was measured as the
216 percentage suppression of endogenous glucose production from basal to the final 30 minutes
217 of the low dose insulin phase (47). Hepatic insulin sensitivity was also quantified during the
218 basal and low dose insulin phase using the hepatic insulin sensitivity index (HISI), which is the
219 reciprocal of the product of endogenous glucose production (glucose Ra) and mean plasma
220 insulin (46).
221 Adipose tissue insulin sensitivity was measured as the percentage suppression of whole body
222 lipolysis (glycerol Ra) from basal to the final 30 minutes of the low dose insulin phase (47).
223 Adipose tissue insulin sensitivity was also quantified during the basal and low dose insulin
224 phase using the adipose tissue insulin sensitivity index (ATIS) which is the reciprocal of the
225 product of whole body lipolysis (glycerol Ra) and basal plasma insulin (46).

226 **Statistical analysis**

227 The Soul-Deep II study was powered on a primary outcome of insulin secretory function (48).
228 Allowing a difference of one standard deviation to be detected with 90% power and 2-sided
229 significance, we aimed to recruit 23 per group, allowing for 20 per group to complete the
230 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

239 and IHL were made using linear multiple regression. Pearson's correlation analyses were used
240 to assess the associations between insulin sensitivity measures and ectopic fat. Interaction by
241 ethnicity was assessed using a linear multiple regression with ethnicity*ectopic fat depot used
242 as the interaction term. Statistical significance was defined as $p < 0.05$ and data analyses were
243 performed using SPSS software, version 25 (IBM Analytics, Armonk, NY, USA).

244 # RESULTS

245 ## Participant characteristics

246 The participant characteristics of the 21 BAM and 23 WEM are displayed in Table 1; by design
247 the groups were comparable in age and BMI. There were no differences in body fat, waist
248 circumference, blood pressure, HbA1c, cholesterol, fasting and post-load glucose between
249 ethnicities; however, BAM exhibited lower fasting triglycerides compared to WEM. Data on
250 ectopic fat depots in these volunteers, as previously reported by our group (39), showed lower
251 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^{-1}, p=0.89) or M/I (BAM:
258 0.0171 (0.0059) vs WEM: 0.0189 (0.0094) ((mg/kg FFM min^{-1}) / (pmol/l)), p=0.44).
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

265 Adipose tissue insulin sensitivity (ATIS) index did not differ by ethnicity during the basal or
266 insulin stimulated state (Table 2). Insulin mediated suppression of glycerol was used as a
267 measure of adipose tissue insulin sensitivity to lipolysis and was not significantly different by
268 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
272 (Figure 1A-C). However, when assessing WEM and BAM separately, adipose tissue insulin
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
282 insulin phase, using PISI (mean difference (95% CI) -1.06 $x10^{-2}$ (-3.87 $x10^{-2}$,1.74 $x10^{-2}$) (µmol
283 /kg FFM min^{-1})/ pmol/l, p=0.43), Table 2. Adjusting PISI for VAT (which we have previously
284 reported as lower in BAM), resulted in significantly lower PISI in BAM (adjusted mean
285 difference (95% CI) -3.47 $x10^{-2}$ (-5.67 $x10^{-2}$, -1.27 $x10^{-2}$) (µmol /kg FFM min^{-1})/ pmol/l,
286 p<0.01). Across the whole cohort, PISI correlated significantly with VAT and IMCL (Figure
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
295 hepatic insulin sensitivity, we found no evidence for an ethnic difference (mean difference
296 (95% CI) -4.15 (-14.83, 6.53) %, p =0.21), Supplementary Figure 2C. Adjusting hepatic insulin
297 sensitivity for VAT, resulted in lower mean hepatic insulin sensitivity in BAM (mean
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-
302 G). When assessing WEM and BAM separately, hepatic insulin sensitivity correlated
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,

332 a so-called lower *threshold*. Our finding of a significant association between VAT and hepatic
333 insulin sensitivity is consistent with earlier work in obese black women (25), however, our data
334 in healthy men also show an association between VAT and skeletal muscle insulin sensitivity
335 which has not been found in women (25, 50). This conflicting result may be due to the
336 aforementioned studies focusing on women with severe obesity whilst our participants were
337 only mildly overweight, or gender itself may explain the conflicting results, adding to the
338 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
349 result (33).

350

351 Intramuscular lipids, which accumulate as a result of skeletal muscle cells taking up fatty acids
352 from the peripheral circulation, have been shown to be correlated with skeletal muscle insulin
353 resistance (15). Whilst we saw a significant relationship between IMCL and skeletal muscle
354 insulin sensitivity in WEM, this relationship was not present in BAM. This finding agrees with
355 other studies (51-53), and suggests that skeletal muscle insulin resistance develops
356 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

376 We have previously published a description of the ectopic fat status for the current set of
377 participants (39) in which we found no ethnic differences in IMCL, but significantly lower
378 VAT and IHL in BAM. In the current study we adjusted our insulin sensitivity data for VAT
379 and found lower whole body, skeletal muscle and hepatic insulin sensitivity in BAM. The
380 reduced insulin sensitivity following adjustment for VAT, and the lower VAT storage in the
381 presence of similar lipolysis, suggests that the detrimental effects of VAT occur at lower levels

382 in BAM and a resistance to storing VAT allows BAM to maintain comparable insulin
383 sensitivity to WEM. In comparison, adjusting for IHL did not explain the similar hepatic insulin
384 sensitivity and provides more evidence for an independent relationship between IHL and
385 hepatic insulin sensitivity in BAM. Lower ectopic fat storage, despite similar lipolysis, may
386 point to an increased tendency towards fat oxidation over ectopic storage in BAM; further
387 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

402 In summary, our data suggest that increased lipolysis due to adipose tissue insulin resistance
403 may not be driving ectopic fat deposition in BAM. Additionally, ectopic fat accumulation in
404 the liver and skeletal muscle may play less of a role in reducing insulin sensitivity in BAM
405 compared to WEM. We provide evidence that the detrimental effects of VAT on glucose uptake
406 and the suppression of endogenous glucose production occur at a lower VAT level in BAM.

407 We conclude that current theories regarding the accumulation of ectopic fat and its impact on
408 insulin sensitivity may not apply in BAM, who display a resistance to storing visceral and
409 hepatic fat. Future work, assessing the impact of ectopic fat on insulin secretory function, is
410 vital before excluding ectopic fat as the culprit behind the increased prevalence of T2D in black
411 populations.

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416 FSM, GCE acquired the data, OB, OH, FSM, AMU performed the data analysis, OB LMG,
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609

610 **Table 1:Participant characteristics**

Characteristic	BAM n=21	WEM n=23	P
Age (years) [a]	25 (22 – 40)	29 (25 – 53)	0.18
BMI (kg/m^2)	26.8 (3.6)	26.5 (4.5)	0.82
Waist Circumference (cm) [b]	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) [b]	70.3 (65.5, 75.5)	70.7 (67.2, 74.3)	0.91
Total Cholesterol (mmol/l) [b]	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) [a]	1.2 (1.2 – 1.4)	1.2 (1.1 – 1.4)	0.86
Triglycerides (mmol/l) [b]	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 (cm^2)[b]	46.1 (34.4 - 61.7)[c]	79.0 (55.4 - 112.5)	**0.02**
Hepatic fat fraction (HFF) (%)	3.78 (1.13)[c]	6.08 (5.04)	**0.04**
Intramyocellular lipid (IMCL) (AU)[d]	0.030 (0.015)	0.030 (0.014)	0.87

611 Data expressed as mean (SD) for normally distributed data
612 [a]data expressed as median (IQR) for non-normally distributed data
613 [b]geometric mean (95% CI) for log transformed data
614 [c]sample size=20
615 [d]sample size; BAM=18, WEM=22

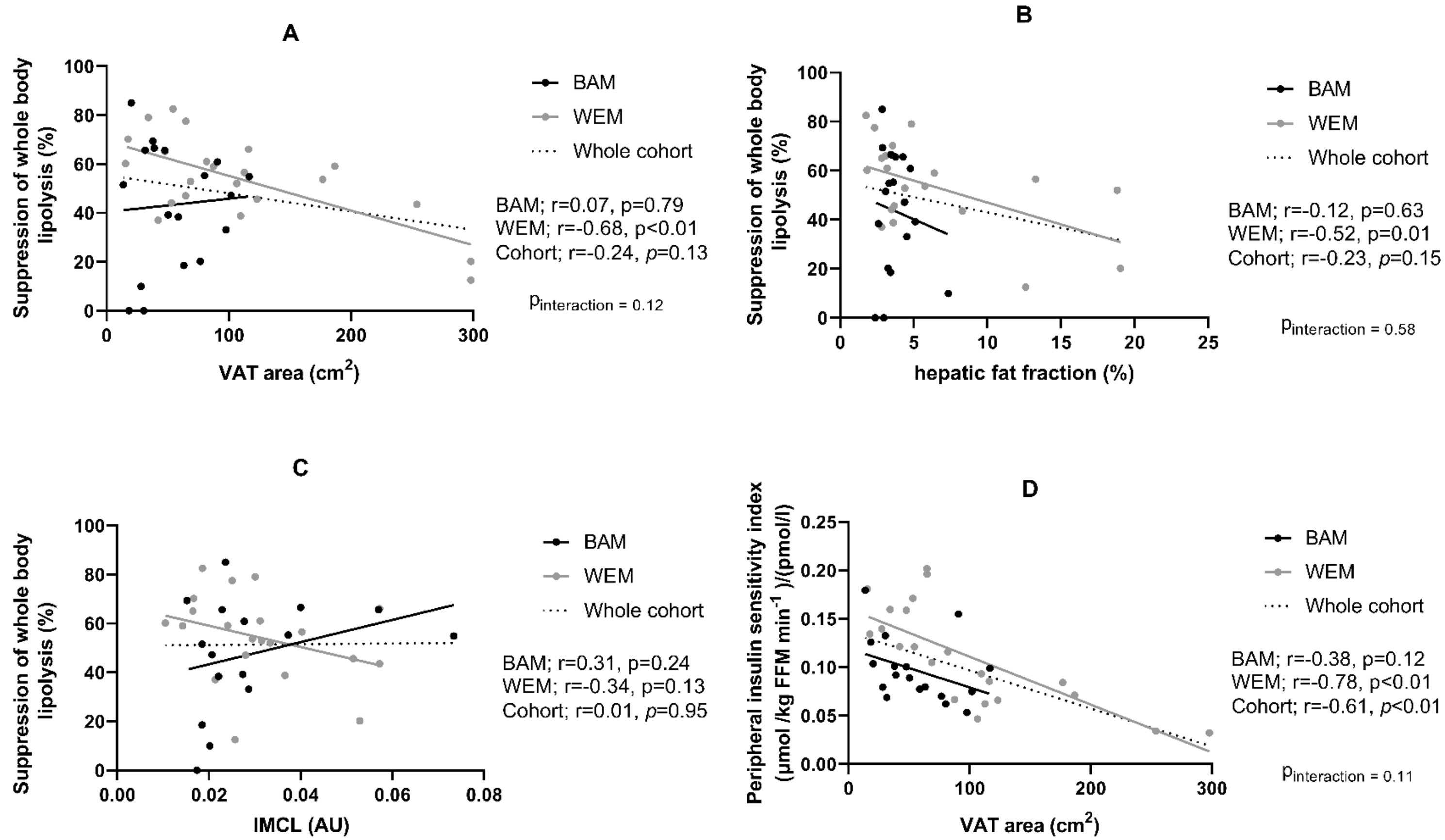
616 **Table 2: Substrate kinetics before and after insulin adjustments during the basal state and the**
617 **hyperinsulinaemic–euglycaemic clamp**

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

Peripheral insulin sensitivity index (µmol /kg FFM min^{-1})/ pmol/l	-	-	-	9.71 x10^{-2} (3.43 x10^{-2})	10.78 x10^{-2} (5.32 x10^{-2})	0.43

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^{-2} BSA min^{-1}). Glucose Rd was derived during the high
620 dose insulin phase (40 mU m^{-2} 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)
623 [a]data expressed as median (IQR)
624 [b]geometric mean (95% CI)
625 [c]sample size; 21
626 [d]sample size; 22
627 Ra = Rate of appearance
628 Rd = Rate of disappearance
629

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



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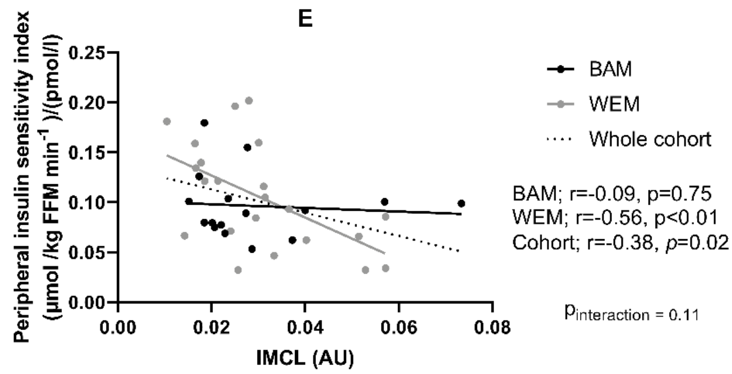

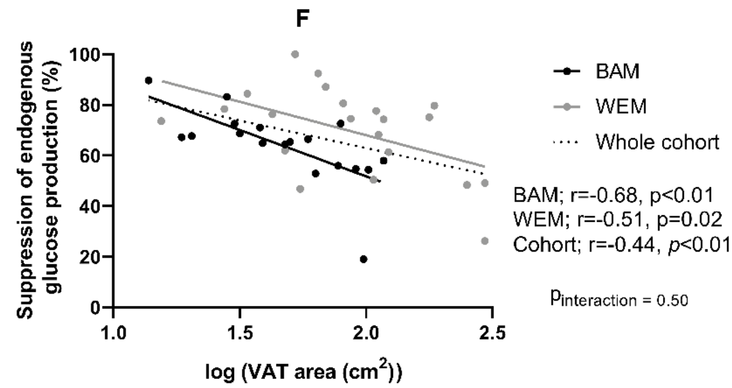

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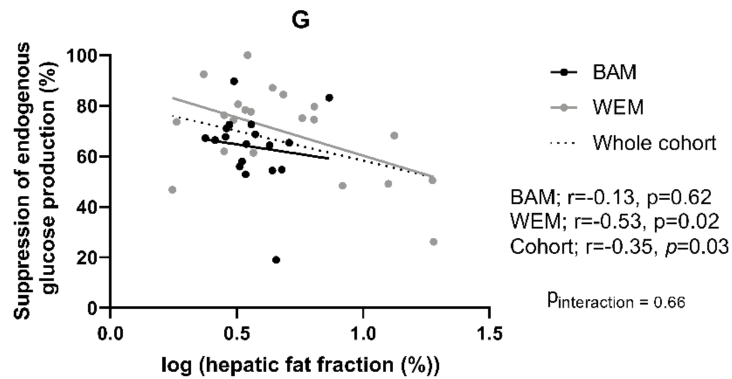


634

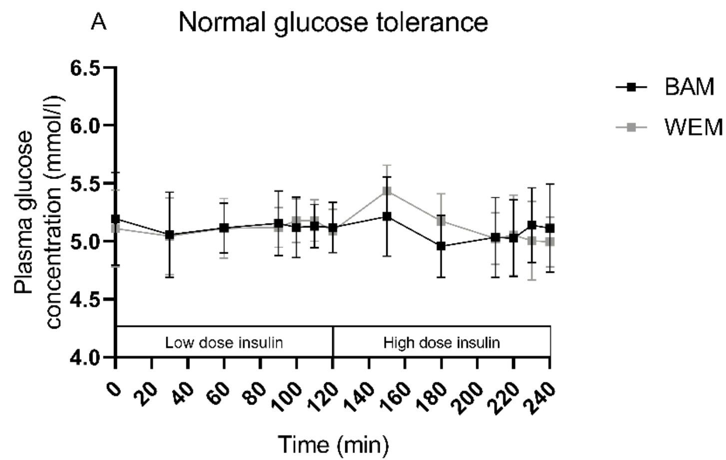
635 Data presented using the Pearson correlation coefficients. Peripheral insulin sensitivity index was measured during the high dose insulin phase
636 (40 mU m^{-2} BSA min^{-1}), suppression of endogenous glucose production and whole body lipolysis was measured during the low dose insulin
637 phase (10 mU m^{-2} BSA min^{-1}).

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

		VAT area (cm^2)	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^{-1}	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;	Whole cohort	**r=-0.63, p<0.01**	**r=-0.61, p<0.01**	**r=-0.40, p=0.01**
((mg/kg FFM min^{-1}) /	BAM	**r=-0.56, p=0.02**	r=-0.34, p=0.17	r=-0.10, p=0.71
(pmol/l))	WEM	**r=-0.78, p<0.01**	**r=-0.73, p<0.01**	**r=-0.54, p=0.01**

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
642

643 Supplementary figure 1: Hyperinsulinaemic–euglycaemic clamp time course



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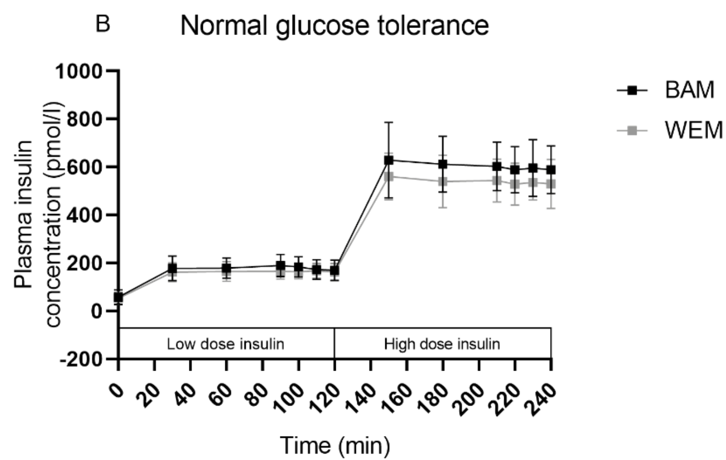
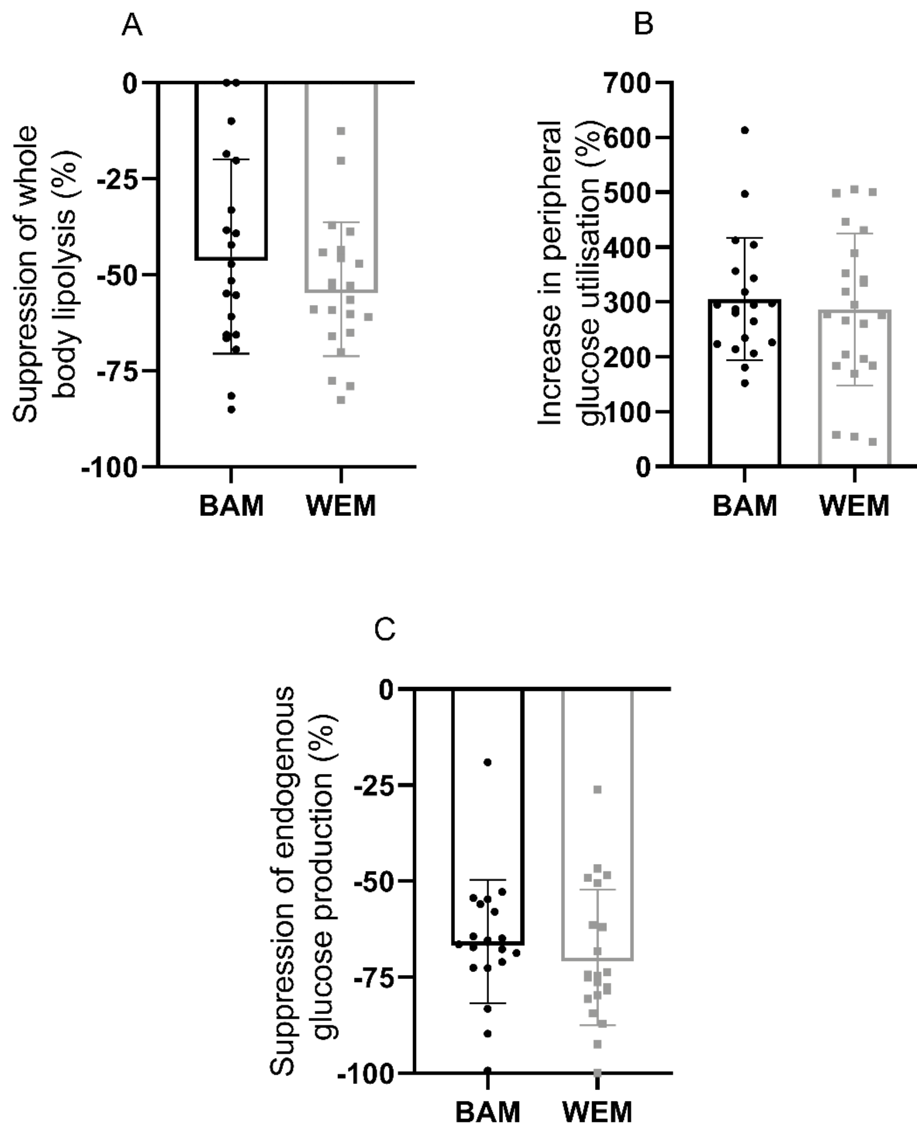


645

646 Data expressed as mean (SEM). Plasma glucose (A) and insulin (B) and concentrations
647 during the hyperinsulinaemic–euglycaemic clamp. Data expressed as Mean ± SD per time
648 point.

649 Supplementary figure 2: Percentage change in glucose and glycerol kinetics during the
650 hyperinsulinaemic–euglycaemic clamp
651



652

653

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