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Ethnic differences in hepatic, pancreatic, muscular and visceral fat deposition in healthy men of white European and black west African ethnicity

Short title: Ectopic fat in men of white and black ethnicity

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Abstract

Aims: We aimed to assess ethnic differences in visceral adipose tissue (VAT), intrahepatic (IHL), intrapancreatic (IPL) and intramyocellular lipids (IMCL) between healthy white European (WE) and black west African (BWA) men.

Methods: 23 WE and 20 BWA men underwent Dixon-magnetic resonance imaging to quantify VAT, IHL and IPL; and proton-magnetic resonance spectroscopy to quantify IMCL. Insulin sensitivity and beta-cell function were determined using homeostasis model assessment (HOMA-2).

Results: BWA men exhibited significantly lower VAT ($P=0.021$) and IHL ($P=0.044$) than WE men, but comparable IPL ($P=0.92$) and IMCL ($P=0.87$). VAT was associated with IPL in both ethnicities (WE: $P<0.001$; BWA: $P=0.001$) but the relationship with IHL differed by ethnicity ($P_{\text{interaction}}=0.018$) and was only significant in WE men (WE: $P<0.001$; BWA: $P=0.36$). All ectopic fat depots inversely associated with insulin sensitivity and positively associated with beta-cell function in WE but not BWA men.

Conclusions: Lower VAT and IHL, and their lack of interrelation, in BWA men suggests ethnic differences exist in the mechanisms of ectopic fat deposition. The lack of association between ectopic fat with insulin sensitivity and beta-cell function in BWA men may indicate a lesser role for ectopic fat in the development of type 2 diabetes mellitus in black populations.

Keywords: Ectopic fat, ethnicity, African, Type 2 diabetes, lipotoxicity

52 **Abbreviations**

53 BWA: black west African

54 BMI: body mass index

55 dSAT: deep subcutaneous adipose tissue

56 EMCL: extramyocellular lipid

57 HbA1c: glycated hemoglobin

58 IHL: intrahepatic lipid

59 IMCL: intramyocellular lipid

60 IPL: intrapancreatic lipid

61 MRI: magnetic resonance imaging

62 MRS: magnetic resonance spectroscopy

63 NAFLD: non-alcoholic fatty liver disease

64 OGTT: oral glucose tolerance test

65 ROI: region of interest

66 SAT: subcutaneous adipose tissue

67 sSAT: superficial subcutaneous adipose tissue

68 T2DM: type 2 diabetes mellitus

69 VAT: visceral adipose tissue

70 WE: white European

Introduction

Ectopic fat, defined as the accumulation of triglycerides in non-adipose tissues, is more strongly related to type 2 diabetes mellitus (T2DM) than measures of whole body adiposity [1, 2]. The advancement of magnetic resonance imaging (MRI) technology for the study of regional adiposity, has confirmed that not all fat is equal and the deposition of ectopic fat has a greater association with insulin resistance than subcutaneous depots which are considered metabolically 'safe' fat storage depots [3-5]. Subsequently, it has been well documented that a central distribution of body fat, more specifically deposition of visceral (VAT) and deep subcutaneous adipose tissue (dSAT), is a major determinant of insulin resistance, while peripheral and superficial subcutaneous adipose tissue (sSAT) depositions are associated with favourable metabolic outcomes [6-8].

There are several theories describing the mechanisms of ectopic fat deposition and their role in the pathophysiology of T2DM. The *spillover theory* hypothesises that the limited expandability of SAT results in an overflow of fatty acids to VAT and dSAT [9-11]. The *portal theory* proposes that the highly lipolytic VAT has a greater flux of fatty acids which are released into the portal circulation and become deposited in the liver (intrahepatic lipid, IHL) [12]. The more recent *twin-cycle theory* proposes that increased IHL accumulation leads to increased export of VLDL-triglyceride from the liver, which subsequently deposit in the pancreas (intrapancreatic lipid, IPL) contributing to the beta-cell failure that underlies the development of frank T2DM [13]. There is evidence showing excess ectopic fat deposition is integral to the development of T2DM by causing metabolic disturbances in the organ/tissue in which it resides, termed *lipotoxicity* [14].

The study of ectopic fat in populations of African ancestry has become an area of increasing interest as despite the greater prevalence of T2DM in black populations compared to white

95 populations they typically have lower levels of ectopic fat [15, 16]. Potential mechanisms of
96 ectopic fat deposition in black populations are poorly understood due to the lack of studies
97 investigating regional adiposity and all ectopic fat depots related to T2DM in a single study.
98 Therefore, by using gold-standard magnetic resonance methods to determine ectopic fat
99 deposition, we aimed to investigate ethnic differences, between healthy white European
100 and black west African men, in 1) VAT, IHL, IPL and intramyocellular lipids (IMCL), 2)
101 relationships between ectopic fat depots and insulin sensitivity and beta-cell function, and
102 3) interrelationships between the ectopic fat depots.

Materials and methods

The South London Diabetes and Ethnicity (Soul-Deep) 2 study is an observational study which aims to investigate ethnic differences in the pathophysiology of T2DM between white European (WE) and black west African (BWA) men [17]. Participants were recruited by local advertisements in the South London area between April 2016 and May 2018. All participants provided written, informed consent and the study was approved by the London Bridge Research Ethics Committee (reference: 15/LO/1121).

Participants

Participants were considered eligible if they were male, aged 18-65 years, had a BMI of 20-40 kg/m², of WE or BWA ethnicity and normal glucose tolerant determined by a 2-hour plasma glucose <7.8 mmol/l during a 75g oral glucose tolerance test (OGTT). Participants were excluded if they had impaired glucose tolerance or T2DM, contraindications for MRI, liver damage determined by a serum alanine aminotransferase of 2.5-fold above the upper limit of reference range, kidney damage determined by serum creatinine of above 150µmol/l or were taking medications known to affect the study outcomes. Eligibility was assessed in a screening assessment including 1) full blood count, renal and liver function, HbA1c, fasting lipid profile and sickle cell trait; 2) anthropometric measurements including height, weight, waist circumference, percentage body fat using bioelectrical impedance (Tanita, MC780) and seated blood pressure; 3) a questionnaire to elicit age; self-declared ethnicity of self, parents and 4 grandparents; medical history and current medications; and 5) a 75g OGTT conducted as previously described to assess glucose tolerance status [18].

The homeostatic model assessment was used to estimate insulin sensitivity (HOMA-S) and beta-cell function (HOMA2-B), which were calculated using the HOMA2 calculator provided

by the Diabetes Trial Unit, University of Oxford (<https://www.dtu.ox.ac.uk/homacalculator/>;
accessed 10/08/19).

Magnetic resonance imaging

Each participant underwent an MRI and magnetic resonance spectroscopy (MRS) scan on a single visit at the Clinical Imaging Department at Guy's Hospital, London. To quantify IHL, IPL, VAT, SAT, dSAT and sSAT each participant underwent a magnetic resonance imaging (MRI) scan on a 1.5 T Siemens Aera scanner. After an overnight fast, participants were scanned between the neck and the knee in the supine position with body coils placed on the scanned area. From each participant, 384 contiguous, axial T1-weighted gradient-echo images (repetition time: 6.77ms; echo times: 4.77ms (in-phase) and 2.39ms (out-of-phase); flip angle: 10°; slice thickness: 3mm) were acquired, from which fat and water images were produced as part of the Dixon sequence. To reduce motion artefacts, participants were instructed to hold their breath for 15 seconds while abdominal images were acquired. MRI data was analysed using HOROS V 1.1.7 (www.horosproject.org; accessed 21/10/2017) by a single analyst who was blinded to clinical data.

Quantification of visceral and subcutaneous adipose tissue

Areas of VAT, SAT, dSAT and sSAT were determined from an axial MRI image at the L4-5 spinal anatomical position. VAT and SAT areas were highlighted to quantify respective areas. DSAT and sSAT were separated from total SAT by locating the fascia-superficialis that is visible on an MRI image.

Quantification of intrapancreatic lipid

IPL was measured using a method we have previously described [19]. Briefly, 3 circular regions of interest (ROI) of approximately 1cm² were positioned on each of the head, body and tail regions of the pancreas; ROIs were checked and approved by a consultant

radiologist (DC) to confirm correct positioning. In each ROI, pancreatic fat fraction was quantified using the formula: $\%IPL = (F/(F+W)) \times 100$, where F is the pixel signal intensity of the fat image and W is the pixel signal intensity of the water image. Mean IPL was calculated as the mean of the head, body and tail IPL.

Quantification of intrahepatic lipid

IHL was measured using a method we have previously described [20]. Briefly, 4 ROIs were positioned in the liver tissue of 2 MRI images while avoiding blood vessels, bile ducts and obvious artefacts. Hepatic fat fraction was calculated in each ROI by using the formula above and IHL was calculated as the mean of all 8 ROIs.

Magnetic resonance spectroscopy

Each participant underwent a ^1H -MRS scan on a 1.5T Siemens system to assess IMCL and EMCL of the soleus muscle of the calf. Participants were scanned in the supine position with their right leg placed within an extremity RF coil to obtain images of the soleus muscle in which a voxel ($1.3 \times 1.3 \times 3.0 \text{ mm}^3$) was positioned while avoiding gross marbling. Localised proton spectra were acquired using a PRESS sequence (TR 2000 ms; TE 30 ms) to obtain two spectra: a water-suppressed lipid spectra and a lipid-suppressed water spectra. The water resonance was set to 4.7ppm, the IMCL resonance was set to 1.3ppm and the EMCL resonance was set to 1.5ppm. MRS spectra were analysed on the Java-Based Magnetic Resonance User Interface (jMRUI) software with the inclusion of prior knowledge to assist in identification of the 4 lipid peaks, methyl and methylene IMCL and EMCL peaks [21]. IMCL and EMCL were expressed in arbitrary units as the ratio of the methylene IMCL or EMCL peaks to internal water.

Statistics

173 The Soul-Deep study included 20 per group to allow a difference of one standard deviation
174 to be detected with power 90% and 2-sided significance for the primary outcome variable
175 (beta-cell insulin secretory function). Non-normally distributed variables were log
176 transformed to achieve a normal distribution. Significance of ethnic differences in variables
177 was tested using an independent samples t-test. Comparison of proportions was conducted
178 using Fisher's exact test. The strength of relationships between fat depots of interest were
179 assessed using Pearson's correlation. The ethnic differences in relationships between fat
180 depots of interest were examined by fitting a regression between the pairs fat depots with
181 an interaction term for ethnicity. All statistical analyses were performed using SPSS 25.0. A
182 p -value <0.05 was considered statistically significant.

Results

Participant characteristics

The clinical characteristics of the 20 BWA and 23 WE normal glucose tolerant men are presented in Table 1. There were no significant ethnic differences in age, weight, BMI, waist circumference, HbA1c, blood pressure and cholesterol levels. Metabolic parameters including fasting insulin, fasting-glucose, 2-hour glucose, HOMA2-S and HOMA2-B also showed no differences by ethnicity. Fasting triglycerides were significantly lower in the BWA men ($p=0.005$).

Visceral and subcutaneous adipose tissue deposition

Data from the analysis of abdominal adipose tissue at the L4-5 position are presented in Fig. 1 and Table 2. There were no significant ethnic differences in abdominal SAT between the two ethnic groups ($p=0.74$). However, VAT was significantly lower in the BWA men compared to the WE men ($p=0.021$), and the ratio of VAT to SAT was also significantly lower in the BWA men ($p=0.038$). There were no significant ethnic differences in dSAT ($p=0.78$), sSAT ($p=0.93$) or the ratio of dSAT to sSAT ($p=0.24$).

Intrahepatic lipid, intrapancreatic lipid and intramyocellular lipid deposition

The IHL, IPL and IMCL data are presented in Fig. 2. The BWA men exhibited significantly lower IHL compared to the WE men ($p=0.044$). Non-alcoholic fatty liver disease (NAFLD), defined as a liver fat level above 5% determined by Dixon-MRI [22], was present in 10% (2/20) of the BWA men compared to 35% (8/23) in the WE men ($p=0.08$). We found no significant ethnic differences in IPL ($p=0.92$); further investigation of regional pancreatic fat deposition showed no significant ethnic differences in IPL in the head ($p=0.86$), body ($p=0.12$) and tail ($p=0.22$) regions of the pancreas, Table 2. MRS data analysis of muscle lipids showed no significant ethnic differences in IMCL ($p=0.76$) or EMCL ($p=0.69$), Table 2.

Interrelationships between the ectopic fat depots

There were ethnic differences in the strength and significance of associations between ectopic fat depots, Fig. 2 and Table 3. VAT was significantly associated with IHL in WE but not BWA men, with a significant ethnicity interaction ($P_{\text{interaction}}=0.018$). VAT was significantly associated with IPL and IMCL in both ethnic groups. IHL was significantly associated with IPL and IMCL in WE men but not BWA men, furthermore, IPL was significantly associated with IMCL in WE men but not BWA men; there were no significant ethnicity interactions for these relationships (all $P_{\text{interaction}} > 0.05$). SAT was significantly associated with VAT and IPL in both ethnic groups, however, the associations between SAT with IHL and IMCL were significant only in WE; a significant ethnicity interaction was present only for the relationship between SAT and IHL ($P_{\text{interaction}}=0.022$).

Relationships between ectopic fat depots and metabolic parameters

Relationships between ectopic fat depots and HOMA2-S and HOMA2-B are presented by ethnicity in Table 4. VAT, IHL, IPL and IMCL were all significantly inversely associated with HOMA2-S and associated with HOMA2-B in WE men but not BWA men.

Discussion

In this study, we have comprehensively assessed ectopic fat deposition in the liver, pancreas and skeletal muscle as well as visceral fat storage in a cohort of well-matched normal glucose tolerant black west African and white European men. We have found lower visceral and hepatic fat in BWA men compared to WE men but no ethnic differences in pancreatic and muscle lipids. While there were strong interrelationships between the ectopic fat depots in the WE men, this was not the case in the BWA men in whom we found no associations between IHL and either VAT or IPL. Additionally, all ectopic fat depots were strongly related to beta-cell function and inversely related to insulin sensitivity in WE men but not BWA men. To our knowledge this is the first study to undertake an ethnic comparison of the four principle ectopic fat depots related to T2DM in a black vs white population.

Our findings of lower VAT and IHL in the BWA men are consistent with earlier studies and provide further evidence for lower VAT and IHL in people of black ethnicity despite their apparently paradoxical increased risk for T2DM [15, 16, 23, 24]. Our data showing a strong association between VAT and IHL in WE men provides evidence to support the portal vein theory which states that VAT is the main driver of IHL, however, this may not be the case in BWA men as we found no association between VAT and IHL in the BWA men. D'Adamo *et al.* previously reported similar findings in adolescents where IHL increased with increasing VAT in whites but not blacks [25]. The ethnic differences in the relationship between VAT and IHL may be explained by ethnic differences in dietary nutrient intake which has been suggested to influence IHL deposition [26, 27]. The associations between SAT and IHL were similar to those of VAT where SAT was associated with IHL in WE but not BWA men. Since both VAT and SAT were not associated with IHL in the BWA men, it appears that the

246 mechanisms of IHL deposition in black populations may differ from white populations and
247 appear to be independent of VAT and SAT deposition.

248 It has been suggested that the deposition of visceral and ectopic fat occurs because of a
249 reduced capacity of SAT to expand, leading to a “spillover” of free fatty acids from the SAT
250 to ectopic depots [1, 9, 11]. Our findings in WE men supports this theory as we reported
251 strong interrelationships between all the ectopic depots in WE men, however, in BWA men
252 other processes may contribute to ectopic fat storage as we reported weaker
253 interrelationships between the depots, except for VAT with IPL and IMCL. Furthermore, our
254 data suggests that organ lipotoxicity and insulin resistance due to ectopic fat storage occurs
255 simultaneously within the muscles and liver resulting from dysfunctional SAT; this process
256 may occur to a greater extent in WE men than BWA men. Supporting the twin-cycle
257 hypothesis that explains IPL deposition, we showed that IHL was associated with IPL in WE
258 men, however, this relationship was not significant in the BWA men indicating that this
259 hypothesis may not occur or occurs to a lesser extent in black individuals.

260 Our data suggest that black individuals have a reduced susceptibility to store VAT as our
261 BWA subjects had similar SAT but lower VAT compared to whites; similar findings have also
262 been reported in black adolescents and adults [25, 28] and we have found the same
263 differences to be present in early T2DM [19]. Our finding of no ethnic differences in dSAT
264 and sSAT in men is matched by previously reported data in black *versus* white men [29].

265 However, in obese populations, blacks have shown greater sSAT deposition with no
266 differences in dSAT compared to their white counterparts indicating a preferential
267 deposition of excess fat in the favourable sSAT depot in black populations with increasing
268 whole-body adiposity [30, 31].

269 We have previously shown that IPL is lower in BWA men compared to WE men with early
270 T2DM [19], however, we found no ethnic differences in IPL in this study of normal glucose
271 tolerant men. This disparity may indicate that WE men have a greater susceptibility to store
272 triglycerides in the pancreas during the development of T2DM. Our findings are supported
273 by an investigation of IPL in black *versus* white/Hispanic women by Lingvay *et al.* who found
274 IPL was significantly lower in black women with T2DM compared to white/Hispanic women
275 but no ethnic differences in IPL were found in the non-diabetic lean or obese women [32]. In
276 our data, VAT was associated with IPL in both WE and BWA men, however, VAT was
277 associated with IHL only in the WE men indicating that the pancreas may be more
278 susceptible to lipid accumulation compared the liver in BWA men.

279 The paradox of greater prevalence of T2DM but lower or similar ectopic fat in black
280 populations compared to white populations indicates that black individuals may be more
281 sensitive to lipotoxicity or that other factors are driving their greater risk of T2DM.

282 Interestingly, whilst we found significant relationships between all measures of ectopic fat
283 and insulin sensitivity (inverse correlations) and beta-cell function in WE men, these were
284 not found in the BWA men, who had comparable insulin sensitivity and beta-cell function.

285 These findings suggest that lipotoxicity by ectopic fat may play a lesser role in the
286 development of T2DM in black populations compared to white populations.

287 Our report of lower IHL in BWA men compared to WE men but no ethnic differences in IPL
288 may indicate that pancreatic lipotoxicity contributes more so to the pathophysiology of
289 T2DM compared to hepatic lipotoxicity in black populations. Toledo-Corral previously
290 showed that IPL predicted prediabetes status in black adolescents but IHL did not [34].

291 However, further investigations of relationships between IHL and IPL with metabolic
292 parameters are required to confirm these speculations.

293 Several studies using data from large cohorts have shown a lower prevalence of NAFLD in
294 black populations [35]. Here we have reported a lower level of NAFLD in the BWA men
295 compared to WE men which was similar to our findings in men with T2DM [20]. An increase
296 of IHL has been linked with the upregulation of hepatic lipogenic processes resulting in
297 hyperlipidaemia [36]. The lower IHL reported in blacks may explain the commonly reported
298 favourable lipid levels in black populations compared with their white counterparts [37, 38].
299 Our finding of no ethnic differences in IMCL supports previous studies conducted in adults
300 and adolescents [30, 39]. In studies investigating the relationships between IMCL and
301 metabolic function, Ingram *et al.* showed IMCL was inversely associated with muscle insulin
302 sensitivity in white adults but not black adults which indicates that IMCL deposition may
303 play a less important role in insulin resistance in black populations [39]. Furthermore,
304 studies comparing ectopic fat deposition between white and black groups have been
305 studied more extensively in adolescents and female populations with a lack of studies being
306 conducted in black men [15, 16, 40].

307 Our study has several strengths worth noting, including the analysis of VAT, IHL and IPL
308 using MRI, which is considered to be the gold-standard non-invasive method to quantify
309 these ectopic fat depots. Our two ethnic groups were well matched for age and BMI,
310 furthermore, we determined metabolic status using an OGTT to include only normal glucose
311 tolerant individuals. Another strength is the inclusion of only men in this study as there is
312 consistent evidence that the pathophysiology of T2DM differs between men and women in
313 both white and black ethnic groups [41]. Furthermore, studies comparing ectopic fat
314 deposition between white and black groups have been studied more extensively in
315 adolescents and female populations with a lack of studies being conducted in black men [15,
316 16, 40].

317 The limitations of this study include the lack of whole body SAT analysis using MRI. Although
318 we have reported percentage whole body fat using bio-electric impedance, this method,
319 which estimates body fat through the use of regression equations, does not account for
320 ethnic variations in body composition [42, 43]. Our study was conducted on a small sample
321 size, although, is comparable in size to other studies conducted on ectopic fat in a black
322 versus white population. Our study lacks data on markers of adipose tissue dysfunction such
323 as pro-inflammatory cytokines which may differ by ethnicity as others have shown [44, 45];
324 hence, investigating these markers may provide insight into the ethnic differences in ectopic
325 fat deposition and is suggested for future studies. Another limitation is that the present
326 study is restricted to a cohort of men with normal glucose tolerance and who may not be
327 destined to develop T2DM. We propose further work must be done to investigate
328 relationships between ectopic fat depots and metabolic parameters in each ethnic group.
329 In conclusion, despite the greater risk of T2DM in black populations, the BWA men exhibited
330 lower levels of visceral adipose tissue and hepatic lipids compared to WE men. Additionally,
331 the lack of association between ectopic fat depots and insulin resistance and beta-cell
332 function in BWA men, which were present in WE men, may indicate that ectopic fat
333 deposition plays a lesser role in the pathophysiology of T2DM in black men compared to
334 white men. Furthermore, the lack of significant interrelationships between ectopic fat
335 depots in BWA men compared to WE men indicates that current theories that describe the
336 mechanisms of ectopic fat deposition may not apply to black populations, which warrants
337 further investigation.

Author contributions

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. O.B. supervised data collection and performed the metabolic assessments. M.L. supervised data collection and performed the metabolic assessments. H.S. conducted MRI data analysis. D.C. conducted MRI data analysis. E.S. conducted MRI data analysis. G.C.E. coordinated MRI data acquisition. O.H. undertook data analysis and interpretation and drafted the manuscript. All authors contributed to the intellectual content and reviewed the final version of the submitted manuscript.

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367 this manuscript.

368 **Table 1: Clinical characteristics of the BWA and WE men**

	BWA (n=20)	WE (n=23)	Mean difference (95% CI)	P
Age (years)	32 (12)	36 (14)	3.6 (-4.5-11.6)	0.38
Weight (kg)	85.0 (13.4)	86.5 (16.5)	1.5 (-7.8-10.8)	0.74
BMI (kg/m ²)	27.0 (3.4)	26.5 (4.5)	-0.42 (-2.9-2.1)	0.74
BMI normal/overweight/obese (n)	6/11/3	11/9/3	-	-
Whole-body fat (%)	20.9 (4.9)	20.9 (6.8)	-0.08 (-3.8-3.6)	0.97
Waist circumference (cm)	88.2 (8.9)	93.8 (14.6)	5.6 (-2.0-13.2)	0.14
Fasting Insulin (pmol/l)	59.1 (30.9)	64.4 (39.9)	5.30 (-16.9-27.5)	0.63
Fasting glucose (mmol/l)	5.14 (0.47)	5.20 (0.39)	0.06 (-0.21-0.32)	0.67
2-hour glucose (mmol/l)	5.22 (1.13)	5.09 (1.26)	-0.13 (-0.87-0.61)	0.72
HOMA2-S	108.0 (45.7)	113.8 (61.2)	5.80 (-27.2-38.8)	0.73
HOMA2-B	93.1 (28.4)	97.5 (37.3)	4.40 (-16.3-25.1)	0.67
HbA1c IFCC (mmol/mol)	37.5 (5.2)	35.9 (2.9)	-1.6 (-4.3-1.0)	0.22
HbA1c DCCT (%)	5.58 (0.47)	5.44 (0.24)	-0.14 (-0.38-0.10)	0.25
Systolic blood pressure (mm/Hg)	122.3 (13.0)	121.9 (9.1)	-0.39 (-7.2-6.5)	0.91
Diastolic blood pressure (mm/Hg)	71.7 (12.0)	71.1 (8.2)	-0.52 (-6.8-5.8)	0.87
Total cholesterol (mmol/l)	4.36 (1.03)	4.76 (1.05)	0.40 (-0.24-1.04)	0.21
LDL cholesterol (mmol/l)	2.71 (0.81)	2.99 (0.82)	0.28 (-0.23-0.78)	0.27
HDL cholesterol (mmol/l)	1.32 (0.45)	1.27 (0.31)	-0.05 (-0.29-0.19)	0.67

	Triglycerides (mmol/l)	0.72 (0.25)	1.10 (0.56)	0.39 (0.12-0.65)	0.005
369	Data presented as mean (SD). Differences between the two ethnic groups were determined using independent samples t-tests.				
370	Abbreviations: BMI, body mass index; BWA, black west African; CI, confidence interval; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; LDL, low				
371	density lipoprotein; WE, white European				

372 **Table 2: Fat deposition in the BWA and WE men**

	BWA (n=20)	WE (n=23)	Mean difference/Ratio of geometric mean (95% CI)	P
Subcutaneous adipose tissue (SAT), L4-5 (cm ²)*	181.9 (136.1-242.9)	193.2 (149.7-249.3)	1.06 (0.73-1.54)	0.74
VAT: SAT, L4-5	0.30 (0.21)	0.47 (0.29)	0.17 (0.01-0.32)	0.038
Deep SAT, L4-5 (cm ²)*	102.6 (72.9-144.6)	109.8 (75.7-159.3)	1.07 (0.65-1.76)	0.78
Superficial SAT, L4-5 (cm ²)*	76.6 (60.3-97.3)	75.5 (61.6-92.6)	0.87 (0.73-1.33)	0.93
Deep SAT: superficial SAT, L4-5	1.42 (0.49)	1.68 (0.84)	0.26 (-0.17-0.69)	0.24
EMCL (AU) ^a	0.031 (0.015)	0.033 (0.018)	0.0027 (-0.008-0.013)	0.61
PFF head (%)*	5.61 (4.40-7.14)	5.46 (4.41-6.76)	0.97 (0.71-1.33)	0.86
PFF body (%)*	6.33 (5.24-7.65)	6.24 (5.11-7.62)	0.99 (0.75-1.29)	0.92
PFF tail (%)*	7.25 (5.92-8.89)	7.56 (6.31-9.04)	1.04 (0.80-1.35)	0.75

373 Data presented as mean (SD) or geometric mean (95% CI) for log transformed data (*). Differences between the two ethnic groups were determined using
374 independent samples t-tests.

375 ^aN for WE=22 and BWA=18

376 Abbreviations: BWA, black west African; CI, confidence interval; EMCL, extramyocellular lipid; HFF, hepatic fat fraction; IMCL, intramyocellular lipid; PFF,
377 pancreatic fat fraction; WE, white European

378 **Table 3: Correlations between measures of regional adiposity and ectopic fat depots in**
379 **WE and BWA men**

		SAT		VAT		IHL		IPL	
		WE	BWA	WE	BWA	WE	BWA	WE	BWA
VAT	<i>r</i>	0.80	0.59	-	-				
	<i>p</i>	<0.001	0.006	-	-				
IHL	<i>r</i>	0.55	0.15	0.67	0.22	-	-		
	<i>p</i>	0.006	0.53	<0.001	0.36	-	-		
IPL	<i>r</i>	0.71	0.59	0.78	0.69	0.85	0.24	-	-
	<i>p</i>	<0.001	0.006	<0.001	0.001	<0.001	0.31	-	-
IMCL	<i>r</i>	0.65	0.15	0.65	0.45	0.42	0.05	0.63	0.15
	<i>p</i>	0.001	0.54	0.001	0.059	0.051	0.86	0.002	0.55

380 Correlation coefficients determined using Pearson's correlation. SAT, VAT, IHL, and IPL were log
381 transformed to achieve a normal distribution. N= 20 BWA and 23 WE for all correlations except for
382 IMCL where n=18 BWA and 22 WE.
383 VAT and SAT determined from an abdominal magnetic resonance image at the L4/5 position; IHL and
384 IPL determined by magnetic resonance imaging; IMCL determined by magnetic resonance
385 spectroscopy.
386 Abbreviations: BWA, black west African; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; IPL,
387 intrapancreatic lipid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WE, white
388 European

389 **Table 4: Correlations between ectopic fat depots and fasting measures of insulin**
390 **sensitivity and beta-cell function in WE and BWA men**

		HOMA2-S		HOMA2-B	
		WE	BWA	WE	BWA
VAT	<i>r</i>	-0.57	-0.37	0.68	0.07
	<i>p</i>	0.003	0.11	0.001	0.77
IHL	<i>r</i>	-0.66	-0.27	0.73	0.34
	<i>p</i>	0.001	0.25	<0.001	0.14
IPL	<i>r</i>	-0.57	-0.11	0.66	-0.19
	<i>p</i>	0.004	0.64	0.001	0.41
IMCL	<i>r</i>	-0.62	-0.06	0.61	-0.14
	<i>p</i>	0.002	0.82	0.003	0.58

391 Correlation coefficients determined using Pearson's correlation. VAT, IHL, IPL and IMCL were log
392 transformed to achieve a normal distribution. N= 20 BWA and 23 WE for all correlations except for
393 IMCL where n=18 BWA and 22 WE.
394 VAT and SAT determined from an abdominal magnetic resonance image at the L4/5 position; IHL and
395 IPL determined by magnetic resonance imaging; IMCL determined by magnetic resonance
396 spectroscopy.
397 Abbreviations: BWA, black west African; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; IPL,
398 intrapancreatic lipid; VAT, visceral adipose tissue; WE, white European

Figure 1: Ectopic fat deposition in white European (WE) and black west African (BWA) men, visceral adipose tissue, (a); hepatic fat fraction, (b); pancreatic fat fraction, (c); intramyocellular lipid, (d). Box and whisker plots show the median and IQ range. Ethnic differences in visceral adipose tissue and hepatic fat fraction were determined using an independent samples t-test.

Figure 2: Relationships between ectopic fat depots in men of white European (WE) and black west African (BWA) ethnicity. Relationships between visceral adipose tissue (VAT) and intrahepatic lipids (IHL), (a); VAT and intrapancreatic lipids (IPL), (b); VAT and intramyocellular lipids (IMCL), (c); IHL and IPL, (d); IHL and IMCL, (e); and IPL and IMCL, (f).

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