RESEARCH: PATHOPHYSIOLOGY

DIABETIC Medicine

Adiponectin is associated with insulin sensitivity in white European men but not black African men

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Abstract

Aims: We aimed to assess ethnic differences in inflammatory markers and their relationships with insulin sensitivity and regional adiposity between white European and black African men.

Methods: A total of 53 white European and 53 black African men underwent assessment of inflammatory markers alongside Dixon-magnetic resonance imaging to quantify subcutaneous and visceral adipose tissue and intrahepatic lipid. A hyperinsulinaemic-euglycaemic clamp was used to measure whole-body and adipose tissue insulin sensitivity. To assess ethnic differences in relationships, the statistical significance of an interaction term between adipokines and ethnic group was tested in multivariable regression models.

Results: The black African men exhibited significantly lower adiponectin and tumour necrosis factor-α (TNF-α) and greater interleukin-10 (IL-10) compared to white European men (all $p < 0.05$). There were no statistically significant ethnic differences in leptin, resistin, IL-6, interferon-γ, IL-13, IL-1β, IL-8 and vascular endothelial growth factor. Several relationships differed significantly by ethnicity such that they were stronger in white European than black African men including IL-6 with visceral adipose tissue; adiponectin with subcutaneous adipose tissue; leptin with intrahepatic lipid; adiponectin, IL-6 and TNF-α with whole-body insulin sensitivity and TNF-α with adipose tissue insulin sensitivity (all $p$interaction <0.05). Leptin significantly predicted whole-body insulin sensitivity in white European ($R^2 = 0.51$) and black African ($R^2 = 0.29$) men; however, adiponectin was a statistically significant predictor in only white European men ($R^2 = 0.22$).

Conclusions: While adiponectin is lower in black African men, its insulin sensitising effects may be greater in white men suggesting that the role of adipokines in the development of type 2 diabetes may differ by ethnicity.

KEYWORDS
adipokine, African, ethnicity, inflammation, type 2 diabetes

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Novelty statement

- Dysregulation of adipokine secretion, ectopic fat and insulin resistance are factors that link adipose tissue dysfunction to type 2 diabetes; however, these are poorly understood in black populations who suffer disproportionately from type 2 diabetes.
- Despite having lower levels of adiponectin, black African men showed favourable inflammatory profiles with lower tumour necrosis factor-α (TNF-α) and greater interleukin-10 (IL-10) compared to white European men.
- Stronger relationships were present between adiponectin, leptin, TNF-α, IL-6 and IL-10 with whole-body and adipose tissue insulin sensitivity in white European men indicating the role of adipokines in type 2 diabetes may differ by ethnicity.

1 | INTRODUCTION

During the development of Type 2 diabetes, the dysfunction of adipose tissue is considered to be one of the key metabolic disturbances that precedes insulin resistance.[1] Adipose tissue dysfunction is characterised by an imbalance in the secretion of key adipokines and cytokines such as adiponectin, leptin, interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) and a high flux of nonesterified fatty acids into the circulation that become deposited as ectopic fat.[2,3] Due to the over secretion of pro-inflammatory cytokines, obesity is recognised as a state of chronic low-grade inflammation which may precede and promote insulin resistance.[2,4] Indeed, epidemiological studies have demonstrated that adiponectin, leptin and IL-6 are independent predictors of Type 2 diabetes.[5,6]

Populations of African ancestry suffer disproportionately high rates of Type 2 diabetes compared to white populations,[7] which may be explained by a greater susceptibility to insulin resistance and hyperinsulinemia.[8–10] Type 2 diabetes develops at lower levels of visceral adipose tissue and intra-hepatic lipid in black populations compared to their white counterparts[9]; this is a paradox unique to black populations since the risk of Type 2 diabetes is typically linked to elevated visceral adipose tissue and ectopic fat accumulation.[11] Investigations of adipokines have consistently shown lower adiponectin and greater leptin levels black compared to white populations even when matched for percentage body fat.[12–15] However, few studies have investigated associations between adipokines, adiposity and insulin sensitivity in a black versus white population. Therefore, we aimed to assess ethnic differences in a range of adipokines implicated in the development of Type 2 diabetes between men of white and black ethnicity. We hypothesised that adiponectin would be (1) lower in black compared to white men, (2) more strongly related to whole-body insulin sensitivity in black than white men and (3) would predict whole-body insulin sensitivity in both ethnic groups.

2 | Participants and methods

This investigation was conducted as part of the South London Diabetes and Ethnicity Phenotyping (Soul-Deep) study which is an observational study designed to investigate ethnic differences in the pathophysiology of Type 2 diabetes between men of white European and black (west) African ethnicity.[16] The Soul-Deep study was reviewed and approved by the London Bridge National Research Ethics Committee (15/LO/1121 and 12/LO/1859). Prior to participation, all participants provided written informed consent. The study was conducted between April 2013 and April 2019.

2.1 | Participants

Men, aged 18–65 years, from three glucose tolerance states (normal glucose tolerance, impaired glucose tolerance and Type 2 diabetes) were targeted by study design for recruitment. Participants were considered eligible if they had a body mass index (BMI) of 20–40 kg/m² (normal glucose tolerant and impaired glucose tolerant) or 25–40 kg/m² (Type 2 diabetes) and were of white European or black (west) African ethnicity. Potential participants underwent a screening assessment to assess eligibility which included a health questionnaire, anthropometric measurements and a fasting blood test. To determine glucose tolerance status, participants underwent a standard 75 g oral glucose tolerance test.[17] Participants were classified as normal glucose tolerant if their 2-h plasma glucose <7.8 mmol/L or impaired glucose tolerant if their 2-h plasma glucose was between 7.8 and 11.1 mmol/L. Glycaemic status of the Type 2 diabetes participants was identified by a recent documented diagnosis of Type 2 diabetes (<5 years) and were eligible if their Type 2 diabetes was treated with lifestyle and/ or metformin only. Participants were excluded from the study if they had contraindications for magnetic resonance imaging and were receiving treatment with thiazolidinedione, insulin, oral steroids, beta-blockers or any other medication that could affect

the study outcomes. Participants were also excluded if they showed evidence of liver or kidney damage, determined from a serum alanine aminotransferase level of 2.5-fold above upper limit of the reference range or serum creatinine level above 150 mmol/L, respectively, or tested positive to anti-insulin, anti-GAD or anti-IA2 auto-antibodies or had sickle cell disease.

2.2 | Study design and procedures

After confirmation of eligibility, participants attended two assessment visits: a hyperinsulinemic-euglycemic clamp carried out at the Clinical Research Facility at King's College Hospital and a magnetic resonance imaging scan carried out at Guy's hospital, London. Prior to the visits, participants were instructed to refrain from (1) strenuous exercise and physical activity for 48 h, (2) alcohol consumption for 24 h and (3) food and drink after 22:00 on the evening before. Participants with Type 2 diabetes on metformin therapy were instructed to cease taking it for 7 days prior to each visit.

2.3 | Magnetic resonance imaging

To assess visceral adipose tissue, subcutaneous adipose tissue and intrahepatic lipid, participants underwent an abdominal magnetic resonance imaging scan using a 2-point Dixon-based magnetic resonance imaging sequence on a 1.5 T Siemens Aera scanner. Abdominal images were acquired during three bouts of 15-s breath-holds. Contiguous axial T1-weighted gradient-echo images (repetition time: 6.77 ms; echo times: 4.77 ms (in-phase) and 2.39 ms (out-of-phase); flip angle: 10°) with a slice thickness of 3 mm were acquired, from which fat and water images were produced. Magnetic resonance imaging data were analysed using HOROS V 1.1.7 (www.horosproject.org; accessed 21/10/2017) by a single analyst who was blinded to clinical data. Areas of visceral adipose tissue and subcutaneous adipose tissue were determined from a single fat-only image at the L4-5 anatomical position where visceral adipose tissue and subcutaneous adipose tissue were highlighted to quantify their respective areas. Intrahepatic lipid was quantified using a previously described method.[18] In brief, for each participant, two abdominal images with a large area of liver tissue were selected. In each corresponding fat and water images, four regions of interest were drawn in identical locations of the liver tissue and the hepatic fat fraction was calculated.

2.4 | Hyperinsulinaemic-euglycaemic clamp

A two-step hyperinsulinaemic-euglycaemic clamp was used to assess whole-body insulin sensitivity.[19] During the clamp,

$[^2H_5]$-glycerol was infused to measure adipose tissue insulin sensitivity. Participants were first administered with a primed (0.12 mg/kg) continuous infusion of $[^2H_5]$-glycerol (rate of infusion: 0.0067 mg kg^{-1} min^{-1}) (CK Gases Ltd) for 240 min in total. After 120 min of the $[^2H_5]$-glycerol infusion, a two-step hyperinsulinaemic-euglycaemic clamp was initiated; participants were administered with a 120-min insulin infusion at a rate of 10 mU/m^2 BSA/min (low dose) followed by 120-min insulin infusion at a rate of 40 mU/m^2 BSA/min (high dose). Throughout the clamp, plasma glucose concentrations were maintained at 5 mmol/L by adjusting the infusion of 20% dextrose according to plasma glucose readings taken every 5 min using an automated glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer). Blood samples were taken at −30, −20, −10, 0, 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230 and 240 min, where time 0 represents the start of insulin low dose infusion, to measure plasma glycerol concentrations and enrichments. Whole-body and adipose tissue insulin sensitivity were calculated as previously described.[20]

2.5 | Biochemical analyses

Inflammatory markers known to be partially or exclusively secreted by adipose tissue were selected for analysis.[21,22] Fasting plasma adipokines and cytokines were determined using immunoassays (Affinity Biomarker Labs). Plasma leptin and total adiponectin were measured using Human Quantikine enzyme-linked immunosorbent assay (ELISA) kits (Bio-Techne). Plasma resistin, TNF-α, interferon-γ (IFN-γ), IL-6, IL-10, IL-13, IL-1β, IL-8 and vascular endothelial growth factor (VEGF) were determined using a Human Proinflammatory multiplex immunoassay kit, Mesoscale Quickplex Discovery SQ120 (Meso Scale Discovery). Plasma glucose concentrations were determined using an automated glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer). Serum insulin was determined by immunoassay using chemiluminescent technology (ADVIA Centaur System; SiemensHealthCare, Ltd). The glycerol enrichment in plasma was determined by gas chromatography-mass spectrometry on Agilent GCMS 5975C MSD (Agilent Technologies) using selected ion monitoring. The isotopic enrichment of plasma glycerol was determined as the tert-butyl trimethylsilyl glycerol derivative.[23]

2.6 | Statistics

The study is part of the secondary outcomes of the Soul-Deep study which included 20 samples per ethnic group with Type 2 diabetes to allow the detection of a difference of one standard deviation with power 90% and two-sided statistical significance

TABLE 1 Clinical characteristics of the black African and white European mens

	Black African, ($n = 53$)	White European, ($n = 53$)	p
Age (years)[a]	40 (36–44)	44 (40–49)	0.15
Weight (kg)	90 ± 14	94 ± 18	0.18
BMI (kg/m^2)	28.8 ± 3.97	29.3 ± 4.92	0.52
Waist circumference (cm)	97 ± 13	100 ± 16	**0.024**
HbA1c IFCC (mmol/mol)	43 ± 8	41 ± 8	0.25
HbA1c NGSP (%)	6.1 ± 0.78	5.9 ± 0.75	0.24
Normal glucose tolerant/impaired glucose tolerant/Type 2 diabetes	23/10/20	23/11/19	–
Fasting glucose (mmol/L)[a]	5.7 (5.5–6.0)	5.8 (5.6–6.1)	0.59
Fasting insulin (pmol/L)[a]	58 (50–67)	61 (48–76)	0.77
Systolic blood pressure (mm Hg)	130 ± 14	130 ± 12	0.26
Total cholesterol (mmol/L)[a]	4.2 (3.9–4.4)	4.6 (4.4–4.8)	**0.011**
LDL-cholesterol (mmol/L)[a]	2.5 (2.3–2.7)	2.7 (2.5–2.9)	0.21
HDL-cholesterol (mmol/L)[a]	1.2 (1.1–1.3)	1.2 (1.1–1.3)	0.40
Triglyceride (mmol/L)[a]	0.87 (0.76–1.0)	1.3 (1.1–1.5)	**<0.001**
Whole-body insulin sensitivity (M-value) (mg/m^2 BSA min^{-1})[b]	240 ± 97	240 ± 130	0.72
Adipose tissue insulin sensitivity (% suppression of lipolysis)[c]	43 ± 21	47 ± 18	0.38
Visceral adipose tissue L4/5 (cm^2)[d]	98 ± 66	150 ± 95	**0.001**
Subcutaneous adipose tissue L4/5 (cm^2)[d]	260 ± 130	260 ± 120	0.99
Intrahepatic lipid (%)[a,e]	4.2 (3.7–4.9)	6.1 (5.0–7.4)	**0.004**

Note: Data presented as mean ± SD for normally distributed data.

Abbreviations: BMI, body mass index; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; LDL, low-density lipoprotein.

[a]Geometric mean (95% CI) for data that were ln-transformed before analysis to achieve a normal distribution. Differences between the two ethnic groups were determined using independent samples t-tests. Bold p-values indicates statistical significance.

[b]N: white European =52, black African =48.

[c]N: white European =43, black African =44.

[d]N: white European =49, black African =50.

[e]N: white European =50, black African =48.

<0.05 in the primary outcome variable (first-phase secretion determined from the hyperglycaemic clamp test). This size of difference is large and of clear clinical importance. Positively skewed variables were transformed using the natural logarithm (ln) to restore symmetry prior to analyses using methods based on the normal distribution. Ethnic differences in mean values of measurements were tested using independent samples t-test. The strength of relationships for each ethnic group, between adipokines and both adiposity and insulin sensitivity indices, was assessed using Pearson's correlation. To determine whether these relations differed between ethnic groups, an interaction term was fitted between adipokines and ethnic group using a multivariable regression model. Factors associated with whole-body insulin sensitivity in each ethnic group were explored using multivariable regression to assess the extent to which the adipokines predicted insulin sensitivity; adiponectin,

leptin, IL-6 and TNF-α were included as independent variables and whole-body insulin sensitivity (M-value) as the dependent variable. Adjustment for BMI and glucose tolerance status was performed by including them separately in the multivariable regression model as covariates. As this study was a secondary analysis and exploratory, we have not adjusted for multiple testing. All statistical analyses were performed using SPSS 25.0, and p-values <0.05 were considered statistically significant.

3 | RESULTS

3.1 | Participant characteristics

We studied 53 black African (23 normal glucose tolerant, 10 impaired glucose tolerant and 20 Type 2 diabetes) and 53 white

European (23 normal glucose tolerant, 11 impaired glucose tolerant and 19 Type 2 diabetes) men. There were no ethnic differences in age, weight, BMI, HbA1c, subcutaneous adipose tissue, whole-body and adipose tissue insulin sensitivity. However, waist circumference, total cholesterol, triglyceride, visceral adipose tissue and intrahepatic lipid were significantly lower in the black African than the white European men (Table 1).

3.2 | Ethnic differences in adipokines

The black African men exhibited significantly lower levels of adiponectin and TNF-α but greater IL-10 than the white European men; however, there were no ethnic differences in leptin, resistin, IL-6, IFN-γ, IL-13, IL-1β, IL-8 and VEGF levels, Table 2.

3.3 | Relationships between adipokines and regional fat depots

Relationships between selected adipokines, known to be related to adipose tissue dysfunction and Type 2 diabetes,[5,21,22] and regional fat depots are presented in Table 3. Adiponectin was significantly inversely associated with subcutaneous adipose tissue in the white European but not the black African men with a statistically significant ethnicity interaction for the relationship between adiponectin and subcutaneous adipose tissue ($p_{interaction} = 0.005$). Leptin was significantly associated with visceral and subcutaneous adipose tissue in both the white European and black African men. However, leptin showed a significantly stronger relationship with intrahepatic lipid in white European men

than black African men ($p_{interaction} = 0.028$). The relationship between IL-6 and visceral adipose tissue differed significantly by ethnicity and was stronger than the white European men than black African men ($p_{interaction} = 0.036$). Additionally, near statistically significant ethnicity interactions were present for the relationships of TNF-α with visceral adipose tissue ($p_{interaction} = 0.056$) and intrahepatic lipids ($p_{interaction} = 0.061$), which were also stronger in white European than black African men.

3.4 | Relationships between adipokines and insulin sensitivity

Relationships between selected adipokines and measures of insulin sensitivity are presented in Table 4 and Figure 1. The relationships of adiponectin, IL-6 and TNF-α with whole-body insulin sensitivity differed significantly by ethnicity ($p_{interaction} = 0.009$, 0.048 and 0.031, respectively) such that they were stronger in the white European than black African men. A statistically significant ethnicity interaction was present for the relationship between TNF-α and adipose tissue insulin sensitivity ($p_{interaction} = 0.020$), which was also stronger in the white European men. Leptin showed a statistically significant association with adipose tissue insulin sensitivity in white European but not black African men with a near statistically significant ethnicity interaction present ($p_{interaction} = 0.060$).

3.5 | Predictors of whole-body adipose tissue

Multivariable regression modelling showed that adiponectin and leptin were both statistically significant predictors of

TABLE 2 Adipokines and inflammatory markers in the black African and white European men

	N (black African, white European)	Black African	White European	Ratio of geometric mean (95% CI)	p
Adiponectin (mg/L)	53, 52	2.6 (2.2–3.0)	3.7 (3.2–4.3)	1.4 (1.2–1.8)	**0.001**
Leptin (μg/L)	47, 51	7.9 (6.5–9.7)	8.0 (6.3–10)	1.0 (0.74–1.4)	0.96
Resistin (μg/L)	52, 53	4.0 (3.6–4.5)	4.0 (3.6–4.4)	0.99 (0.85–1.2)	0.94
IL−6 (ng/L)	53, 53	0.93 (0.82–1.1)	1.0 (0.90–1.2)	1.1 (0.92–1.3)	0.29
TNF-α (ng/L)	51, 53	2.4 (2.2–2.5)	2.7 (2.5–2.9)	1.1 (1.0–1.3)	**0.009**
IFN-γ (ng/L)	36, 51	4.7 (4.0–5.6)	5.0 (4.4–5.6)	1.1 (0.86–1.3)	0.59
IL−10 (ng/L)	51, 53	0.62 (0.56–0.70)	0.50 (0.45–0.56)	0.80 (0.68–0.94)	**0.007**
IL−13 (ng/L)	42, 42	1.6 (1.3–1.9)	1.5 (1.3–1.8)	0.97 (0.77–1.2)	0.80
IL−1β (ng/L)	35, 27	0.16 (0.13–0.19)	0.15 (0.11–0.19)	0.95 (0.70–1.3)	0.71
IL−8 (ng/L)	51, 53	8.8 (8.0–9.7)	8.5 (7.6–9.5)	0.97 (0.84–1.1)	0.66
VEGF (ng/L)	53, 53	70 (57–87)	60 (50–73)	0.86 (0.65–1.1)	0.30

Note: Data presented as geometric mean (95% CI). Differences between the two ethnic groups were determined using independent samples *t*-tests. All data were ln-transformed before analysis to achieve a normal distribution. Bold *p*-values indicate statistical significance.

Abbreviations: CI, confidence interval; IFN, interferon; IL, interleukin; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

TABLE 3 Correlations between adipokines and inflammatory markers with regional fat depots in the white European and black African men

	Visceral adipose tissue		Subcutaneous adipose tissue		Intrahepatic lipid[a]	
	White European	Black African	White European	Black African	White European	Black African
Adiponectin[a]						
r	−0.22	−0.20	**−0.35**	0.12	**−0.31**	−0.22
95% CI	−0.47 to 0.007	−0.47 to 0.11	−0.61 to -0.064	−0.17 to 0.40	−0.58 to -0.071	−0.48 to 0.088
p	0.13	0.16	**0.014**	0.40	**0.028**	0.14
n	49	50	49	50	50	48
Leptin[a]						
r	**0.62**	**0.56**	**0.81**	**0.82**	**0.69**	**0.41**
95% CI	0.39–0.79	0.36–0.73	0.69–0.88	0.70–0.90	0.51–0.82	0.092–0.62
p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.006**
n	48	46	48	46	49	44
IL-6[a]						
r	**0.55**	**0.29**	**0.38**	0.22	**0.48**	**0.39**
95% CI	0.32–0.72	0.044–0.50	0.16–0.58	−0.022 to 0.47	0.24–0.71	0.12–0.62
p	**<0.001**	**0.039**	**0.007**	0.12	**<0.001**	**0.006**
n	49	50	49	50	50	48
TNF-α[a]						
R	**0.30**	0.11	**0.37**	0.24	**0.42**	0.17
95% CI	−0.031 to 0.60	−0.21 to 0.36	0.14 to 0.57	−0.16 to 0.51	0.18–0.61	−0.10 to 0.41
p	**0.035**	0.44	**0.010**	0.10	**0.002**	0.27
n	49	48	49	48	50	46
IL-10[a]						
r	0.003	−0.14	−0.03	−0.15	0.09	−0.13
95% CI	−0.24 to 0.30	−0.45 to 0.12	−0.22 to 0.24	−0.36 to 0.080	−0.12 to 0.32	−0.43 to 0.15
p	0.98	0.33	0.83	0.30	0.54	0.39
n	49	48	49	48	50	46

Note: Correlation coefficients determined using Pearson's correlation. Bold r and p-values indicates statistical significance.

Abbreviations: CI, confidence interval; IL, interleukin; TNF, tumour necrosis factor.

[a]Data were ln-transformed before analysis to achieve a normal distribution.

whole-body insulin sensitivity in the white European men, while in the black African men, only leptin was a statistically significant predictor; the four predictor model, which included adiponectin, leptin, IL-6 and TNF-α as independent variables, accounted for 56% of the variance in insulin sensitivity in the white European men and 31% in the black African men. After adjustment for glucose tolerance status, there were no appreciable changes in the estimates. After adjustment for BMI, the relationship between leptin and whole-body insulin sensitivity diminished in both ethnic groups; however, that of adiponectin remained in the white European men. Modelled individually, leptin accounted for 51% of the variability in whole-body insulin sensitivity in the white European men and 29% in the black African men, while adiponectin accounted for 22% of the variability in white European men but only 2% in the black African men.

4 | DISCUSSION

In this study, we have shown ethnic differences in adipokine levels and the relationships between key adipokines and measures of adiposity and insulin sensitivity between men of white European and black African ethnicity. Despite no ethnic differences in whole-body adiposity or insulin sensitivity, black African men exhibited lower adiponectin and TNF-α levels, while IL-10 was higher compared to white European men. Additionally, exploratory correlation analysis showed that stronger relationships were present between adipokines and measures of insulin sensitivity in white European men than black African men, in whom the only statistically significant relationship was between leptin and whole-body insulin sensitivity; however, this relationship diminished after adjustment for BMI. In contrast to our hypothesis, adiponectin

TABLE 4 Correlations between adipokines and inflammatory markers with measures of fasting insulin and insulin sensitivity in the white European and black African men

	Fasting insulin[a]		Whole-body insulin sensitivity (*M*-value)		Adipose tissue insulin sensitivity	
	White European	Black African	White European	Black African	White European	Black African
Adiponectin[a]						
r	**−0.37**	**−0.32**	**0.48**	0.15	**0.33**	0.13
95% CI	−0.60 to −0.11	−0.54 to −0.088	0.27–0.65	−0.12 to 0.41	−0.021 to 0.62	−0.19 to 0.41
p	**0.009**	**0.019**	**<0.001**	0.31	**0.033**	0.40
n	50	52	51	48	42	44
Leptin[a]						
r	**0.61**	**0.33**	**−0.72**	**−0.55**	**−0.46**	−0.11
95% CI	0.44–0.76	0.069–0.55	−0.86 to −0.55	−0.72 to −0.32	−0.69 to −0.18	−0.40 to 0.20
p	**<0.001**	**0.026**	**<0.001**	**<0.001**	**0.003**	0.49
n	49	46	50	43	41	39
IL-6[a]						
r	**0.51**	0.06	**−0.46**	−0.15	−0.26	0.06
95% CI	0.28–0.72	−0.29 to 0.39	−0.64 to −0.24	−0.44 to 0.15	−0.50 to 0.003	−0.23 to 0.31
p	**<0.001**	0.68	**0.001**	0.31	0.092	0.70
n	51	52	52	48	43	44
TNF-α[a]						
r	**0.57**	**0.28**	**−0.34**	−0.02	**−0.39**	0.03
95% CI	0.41–0.70	0.041–0.49	−0.52 to −0.11	−0.33 to 0.28	−0.65 to −0.093	−0.26 to 0.31
p	**<0.001**	**0.049**	**0.015**	0.91	**0.010**	0.83
n	51	50	52	46	43	42
IL-10[a]						
r	−0.005	−0.24	0.01	0.16	0.28	0.12
95% CI	−0.22 to 0.27	−0.49 to 0.091	−0.23 to 0.34	−0.11 to 0.45	−0.070 to 0.53	−0.16 to 0.40
p	0.97	0.091	0.64	0.29	0.065	0.43
n	51	50	52	46	43	43

Note: Correlation coefficients determined using Pearson's correlation. Bold *r* and *p*-values indicates statistical significance.

Abbreviations: CI, confidence interval; IL, interleukin; TNF, tumour necrosis factor.

[a]Data were ln-transformed before analysis to achieve a normal distribution.

was associated with whole-body insulin sensitivity in white European but not black African men.

Our study supports previous studies that have shown lower levels of adiponectin in black populations compared to their white counterparts.[24,25] Adiponectin is exclusively secreted by adipose tissue and has protective anti-inflammatory and insulin sensitising properties.[26] The lower levels of adiponectin present in black populations have been proposed to negatively affect insulin sensitivity and contribute to the onset of Type 2 diabetes in blacks. However, in contrast to this belief, our data suggest that adiponectin is not associated with insulin sensitivity in black African men. Therefore, even though adiponectin levels were lower in the black African men, it may not play an integral role in reducing insulin sensitivity in black populations. Our findings support those of Farris et al.

who reported an association between adiponectin and insulin sensitivity in white but not black individuals.[25] Additionally, Evans et al. showed that the subcutaneous adipose tissue inflammatory gene expression of black women explained a small proportion (20%) of their variation in insulin sensitivity in comparison to 56% in white women.[27] The lack of relationship between adiponectin and insulin sensitivity in black populations may be more prominent in men since findings from the Jackson Heart Study showed that adiponectin was significantly associated with Type 2 diabetes in black women but not black men[28]; this suggests that the role of adiponectin in Type 2 diabetes may differ by gender in black populations.

Even though the black African men exhibited lower levels of adiponectin, they showed signs of more favourable inflammatory profiles compared to the white European men as indicated
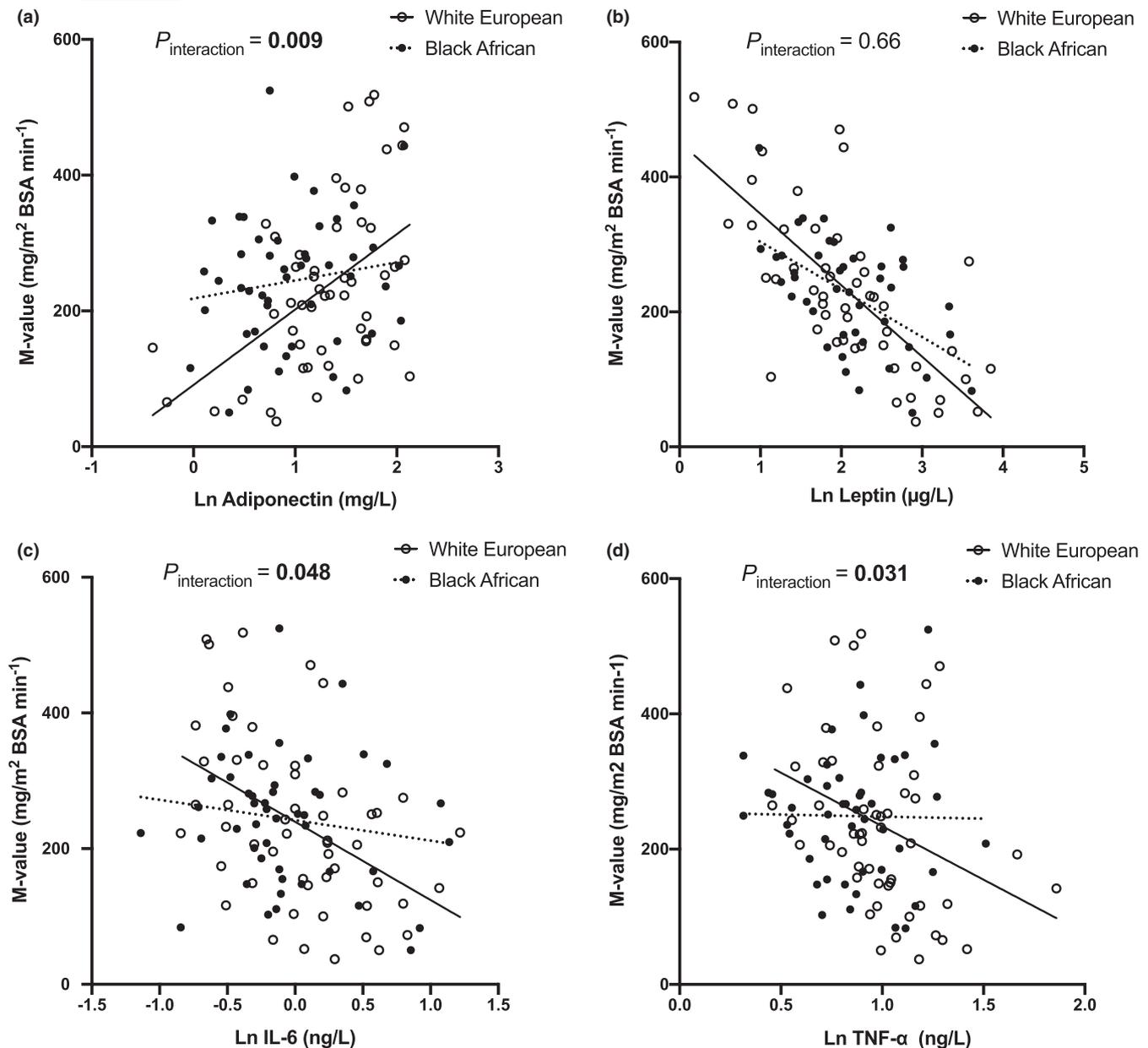
FIGURE 1 Relationships between adipokines and whole-body insulin sensitivity (*M*-value) in men of white European and black African ethnicity. Relationships between whole-body insulin sensitivity (*M*-value) and adiponectin (a), leptin (b), interleukin-6 (IL-6) (c) and tumour necrosis factor-α (TNF-α) (d). Adiponectin, leptin, IL-6 and TNF-α underwent ln transformation to achieve a normal distribution

by lower TNF-α and greater IL-10. The lower levels of TNF-α that we found in the black African men may be explained by significantly lower levels of visceral adipose tissue present in the black African men since it has been shown that TNF-α is highly expressed in visceral adipose tissue.[29] In support of our findings, Hyatt et al. showed that not only was TNF-α lower in African American women in comparison to BMI-matched white women but the two cell-surface receptors for TNF-α, sTNFR-1 and sTNFR-2 were also lower.[30] Furthermore, also similar to our findings, Hyatt et al. showed that TNF-α was related to visceral adipose tissue in white women but not African American women.[30] Hence, the pro-inflammatory response of the visceral adipose tissue depot may differ by ethnicity.

In agreement with existing literature, leptin showed strong associations with all measures of adiposity in both ethnic groups.[15] However, the inverse associations we found, in both ethnic groups, between leptin and whole-body insulin sensitivity diminished after adjustment for BMI suggesting that they may be a direct reflection of the relationship between adiposity and insulin resistance. In contrast, the relationship between adiponectin and whole-body insulin sensitivity remained after adjustment for BMI in the white European men; this may indicate that while leptin appears to be a marker of adiposity, adiponectin may play a protective role by increasing insulin sensitivity, more prominently so in white European men.

A novel aspect of this study is the investigation of the impact of ethnicity on the relationships between adipokines, adipose tissue insulin sensitivity and ectopic fat in a single study; we showed ethnic distinctions in the relationships between these three key markers of adipose tissue dysfunction. Both leptin and TNF-α were more strongly related to intrahepatic lipid deposition and adipose tissue insulin sensitivity in the white European men than black African men. The stronger relationships between adipokines, adipose tissue insulin sensitivity and intrahepatic lipid levels in the white European men may suggest that the role of adipose tissue dysfunction in the development of Type 2 diabetes may differ by ethnicity. In addition, the markers of adipose tissue dysfunction and the mechanisms by which they contribute to insulin resistance ought to be investigated further in black ethnic groups. Undoubtedly, a greater understanding of adipose tissue inflammation in Type 2 diabetes in black populations will inform future ethnic-specific strategies for the prevention and treatment of Type 2 diabetes.

This study has several strengths including the analysis of several adipokines as well as the assessment of whole-body and adipose tissue insulin sensitivity using the hyperinsulinemic-euglycemic clamp and regional adiposity using magnetic resonance imaging. To our knowledge, this is the first study to assess relationships between inflammatory markers and adipose tissue insulin sensitivity in a black versus white population. Our study has some limitations including the cross-sectional nature of the study which does not allow us to determine causality between adipokines and measures of insulin sensitivity. The small sample size available means that only large differences in the mean of one standard deviation or more could be detected and so some real differences may have been missed. However, in contrast, we can be reasonably confident that where significant interactions were observed, a real and large clinically important difference was shown. Our study is a secondary exploratory analysis, so we have not adjusted for multiple testing. Hence, our findings need replicating in other datasets. Another limitation is the lack of whole-body subcutaneous adipose tissue data using multiple slice magnetic resonance imaging data. Although we have reported abdominal subcutaneous adipose tissue area, this was determined using a single magnetic resonance imaging slice, which may not reflect ethnic differences in whole-body subcutaneous adipose tissue. The generalisability of our findings is limited due to the inclusion of only men in our study; however, previous studies have mostly focused on black women due to their greater levels of obesity while studies comparing black and white men are lacking in this field. Furthermore, some studies suggest that the development of Type 2 diabetes may be more strongly driven by excess adiposity in black women than black men, which warrants investigation into the impact of gender in the pathophysiology of Type 2 diabetes in black populations.[31,32]

To conclude, our exploratory analysis of markers of adipose tissue inflammation suggests that the role of adipokines in the development of Type 2 diabetes may differ between black and white men. Leptin was associated with whole-body insulin sensitivity in both white European and black African men, but these relationships diminished after adjustment for BMI, suggesting that leptin is merely a marker of adiposity. However, adiponectin was associated with whole-body insulin sensitivity in the white European men but not the black African men, a relationship which was independent of glucose tolerance and BMI indicating that the insulin sensitizing effects of adiponectin may be greater in white men than black men. Overall, our study suggests that adipokine secretion does not appear to explain the greater risk of insulin resistance in black populations and further studies are required to elucidate ethnic differences in the mechanisms of adipokine action.

CONFLICT OF INTEREST
The authors declare that there is no duality associated with this manuscript.

AUTHOR CONTRIBUTIONS
Study design and formulation of the research question: L.M.G., S.A.A., A.M.U., J.L.P.; Data collection: O.B., M.L., G.C.E., L.M.G., C.M.; Data analysis and interpretation: O.H., F.S., N.J., L.M.G., A.M.U., J.L.P.; Manuscript drafting: O.H.; All authors contributed to the intellectual content and reviewed the final version of the submitted manuscript.

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