RESEARCH

β-cell function in black South African women: exploratory associations with insulin clearance, visceral and ectopic fat

Melony C Fortuin-de Smidt[1,2], **Amy E Mendham**[1,2], **Jon Hauksson**[3], **Ali Alhamud**[4,5], **Darko Stefanovski**[6], **Olah Hakim**[7], **Jeroen Swart**[1], **Louise M Goff**[7], **Steven E Kahn**[8], **Tommy Olsson**[9] and **Julia H Goedecke**[1,2]

[1]Division of Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town, Cape Town, South Africa
[2]Non-Communicable Diseases Research Unit, South African Medical Council, Tygerberg, South Africa
[3]Department of Radiation Sciences, Radiation Physics and Biomedical Engineering, Umea University, Umea, Sweden
[4]Department of Human Biology, MRC/UCT Medical Imaging Research Unit, University of Cape Town, Cape Town, South Africa
[5]The Modern Pioneer Center and ArSMRM for MRI Training and Development, Tripoli, Libya
[6]Department of Clinical Studies, New Bolton Centre, University of Pennsylvania School of Veterinary Medicine, Kennett Square, Pennsylvania, USA
[7]Department of Diabetes, Faculty of Life Sciences and Medicine, School of Life Course Sciences, King's College London, London, UK
[8]Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, Veterans Affairs Puget Sound Health Care System, University of Washington, Seattle, Washington, USA
[9]Department of Public Health and Clinical Medicine, Umea University, Umea, Sweden

Correspondence should be addressed to M C Fortuin-de Smidt: mcfortuindesmidt@gmail.com

Abstract

The role of ectopic fat, insulin secretion and clearance in the preservation of β-cell function in black African women with obesity who typically present with hyperinsulinaemia is not clear. We aim to examine the associations between disposition index (DI, an estimate of β-cell function), insulin secretion and clearance and ectopic fat deposition. This is a cross-sectional study of 43 black South African women (age 20–35 years) with obesity (BMI 30–40 kg/m^2) and without type 2 diabetes that measured the following: DI, insulin sensitivity (S_I), acute insulin response (AIRg), insulin secretion rate (ISR), hepatic insulin extraction and peripheral insulin clearance (frequently sampled i.v. glucose tolerance test); pancreatic and hepatic fat, visceral adipose tissue (VAT) and abdominal s.c. adipose tissue (aSAT) volume (MRI), intra-myocellular (IMCL) and extra-myocellular fat content (EMCL) (magnetic resonance spectroscopy). DI correlated positively with peripheral insulin clearance (β 55.80, P = 0.002). Higher DI was associated with lower VAT, pancreatic fat and soleus fat, but VAT explained most of the variance in DI (32%). Additionally, higher first phase ISR (P = 0.033) and lower hepatic insulin extraction (P = 0.022) were associated with lower VAT, independent from S_I, rather than with ectopic fat. In conclusion, peripheral insulin clearance emerged as an important correlate of DI. However, VAT was the main determinant of a lower DI above ectopic fat depots. Importantly, VAT, but not ectopic fat, is associated with both lower insulin secretion and higher hepatic insulin extraction. Prevention of VAT accumulation in young black African women should, therefore, be an important target for beta cell preservation.

Key Words

- β-cell function
- hepatic insulin extraction
- peripheral insulin clearance
- insulin secretion
- ectopic fat

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Introduction

The acute insulin response (AIRg) relative to the prevailing level of insulin sensitivity (S_I) described by the disposition index (DI) estimates the β-cell function, which is a key component in the pathogenesis of type 2 diabetes (T2D) (1). The primary events in the development of T2D may differ between black Africans and their white counterparts. Indeed, black Africans without T2D present with lower S_I and hyperinsulinaemia, characterized by greater fasting insulin and AIRg in comparison to white individuals (2). In some cases, the AIRg response is exaggerated for the level of S_I (3), resulting in a higher DI. Determinants of S_I in black African populations have been studied extensively but there is limited evidence on the determinants of hypersinsulinaemia and its components, namely insulin secretion rate (ISR) and insulin clearance.

A lower hepatic insulin extraction (FE_L) is found in black African women (4) which contributes towards a high AIRg (5), but how it relates to DI is unknown. In addition, the relative contribution of insulin clearance and insulin secretion to a high AIRg has only been reported in African–American children (5), but studies in black African adults with obesity and without T2D are lacking. Further, while the liver is the main organ involved in insulin clearance and clears approximately 50–70% of insulin during the first pass through the liver (6), insulin is also cleared by peripheral tissue such as the kidneys, muscle and adipose tissue, amongst others. However, the association of peripheral insulin clearance (CLp) with β-cell function needs further clarification.

Few studies have assessed associations between measures of β-cell function and VAT (7, 8, 9) and ectopic fat deposition (10, 11) in African–Americans, but no studies have been undertaken in an African cohort, which is required to determine if this phenotype is robust. Indeed, studies from Africa and the USA have consistently shown that black populations present with lower ectopic fat accumulation compared to white populations (10, 12, 13), together with hyperinsulinaemia (2) and paradoxically a higher prevalence of T2D. Whether black African populations exhibit a greater sensitivity to ectopic fat accumulation or whether ectopic fat is not an important correlate of β-cell function and its components requires further elucidation. Accordingly, this study aimed to evaluate the correlates of β-cell function, assessed by DI, focusing on hyperinsulinaemia (insulin secretion, FE_L and CLp) and its association with ectopic fat depots in black South African women with obesity.

Materials and methods

Research design

This study was a cross-sectional analysis of baseline data obtained from a randomized, controlled exercise intervention study, for which the study design and methods have been published previously (14). Participants were recruited from low socio-economic areas in Cape Town, South Africa. Ethical permission was obtained from the University of Cape Town Human Research Ethics Committee. The trial was registered in the Pan African Clinical Trial Registry (PACTR201711002789113).

Participants

Black African (self-reported ancestry with both parents of isiXhosa descent) women with obesity (BMI 30–40 kg/m^2) that fulfilled the following inclusion criteria were enrolled: age 20–35 years, stable weight (<5 kg change in weight in the last 6 months), using injectable contraception (depot medroxyprogesterone acetate, 400 mg) for a minimum of 2 months prior to testing. Different types of hormonal contraception (injectables compared to oral contraception) have differential effects on fasting glucose and insulin levels (15). In Khayelitsha, where most of our participants reside, 83% of women use injected contraceptives and in the majority (75%), the method of contraception is determined by the healthcare provider (16). Therefore, to minimize the effect of hormonal fluctuations on our study findings, our inclusion criteria ensured participants were all using the same contraceptive method based on the standard of care in the public health system. Participants were excluded if they had any known diseases (e.g. HIV (HIV rapid negative), hypertension (BP ≥ 140/90 mmHg), diabetes (HbA1c > 6.5%), anaemia (haemoglobin <12 g/dL)), pregnant, lactating or smoking. After written informed consent was obtained, patients were screened for inclusion.

Body composition

Anthropometry included weight, height, hip and waist circumferences were measured using standardized methods. Whole-body composition was measured by dual-energy X-ray absorptiometry (DXA; Discovery-W®, software version 12.7.3.7; Hologic, Bedford, MA) for the analyses of sub-total body (whole body minus head) fat mass and fat-free soft tissue mass, as well as trunk and leg mass (expressed as per cent of sub-total body fat mass) (17).

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Ectopic and central fat quantification

Pancreatic, hepatic and skeletal muscle (soleus, tibialis anterior) fat content, VAT and abdominal s.c. adipose tissue (aSAT) volumes were analysed using a 3 Tesla whole-body human MRI scanner (MAGNETOM Skyra; Siemens Medical Solutions) using a two-point Dixon method as described previously (14). Soleus and tibialis anterior intra-myocellular (IMCL) and extra-myocellular (EMCL) fat content were determined by magnetic resonance spectroscopy (MRS) using a point-resolved spectroscopy sequence with the following parameters: TR/TE 3000/33 ms, bandwidth 2000 Hz, 80 averages with voxel dimensions of 15 × 15 × 15 mm^3.

For the determination of hepatic, pancreatic and skeletal muscle fats, VAT and aSAT obtained by MRI, a fat fraction map was created and calculated as the fat signal over the sum of the water and fat signals. A region of interest (ROI) was drawn on seven consecutive slices in the right lobe of the liver. In the soleus and tibialis anterior muscles, an ROI was drawn on seven consecutive slices with a method adapted from Machann *et al.* (18). Pancreatic fat was determined by drawing one circular 1 cm^2 ROI in the head, body and tail of the pancreas to calculate the average (19). The methods for determining VAT and aSAT volumes were previously published (20). The above-mentioned methods, to quantify hepatic (21), pancreatic (22) and skeletal muscle fat (23) and VAT and aSAT (24), have previously been reported to have high precision.

Determination of S_I and β-cell function

An FSIGT allows for the determination of the acute insulin response after an i.v. glucose load (25). After 20 min, insulin is infused to suppress the endogenous insulin production which enables the evaluation of the effect of insulin on glucose disappearance. An FSIGT was used in this study because it provides measures of the AIRg as well as S_I and it is less labour intensive compared to the clamp method and does not require a steady-state. Additionally, the FSIGT shows a good correlation to the hyperglycaemic clamp (26). The FSIGT was conducted after an overnight fast (10–12 h). Glucose (50% dextrose, 11.4 g/m^2 body surface area) was infused at 0 min over a 60 s period. At 20 min, human insulin (0.02 unit/kg; NovoRapid, Novo Nordisk Limited) was infused over 5 min at a constant rate. Thirty-two blood samples were drawn over 240 min for the determination of plasma glucose and serum insulin and C-peptide.

Biochemical analyses

Fasting whole blood was collected and analysed for HbA1c using HPLC (Meharini Diagnostics, Florence, Italy). Plasma glucose concentrations were measured using a colorimetric assay (Randox (Pty) Ltd, Gauteng, South Africa). Serum insulin and C-peptide concentrations were determined by an immunochemiluminometric assay (IMMULITE 1000 immunoassay system, Siemens Healthcare (Pty) Ltd).

Mathematical modelling

The minimal model of glucose kinetics was used to calculate S_I, AIR_g and glucose effectiveness (27). S_I is a measure of the fractional disappearance of glucose for a given insulin concentration achieved by uncoupling the glucose and insulin responses. Glucose effectiveness is a measure of the ability of glucose to enhance its own uptake. AIRg is the incremental area under the insulin curve in response to i.v. glucose over the first 10 min and reflects the first phase insulin response. DI is a measure of the insulin response to glucose relative to the prevailing level of S_I (AIRg × S_I) which gives an index of whether the insulin response is adequate for the level of S_I (1). The ISR was determined by C-peptide deconvolution using a 2-compartmental model with standard kinetic parameters for individuals with obesity (28). The incremental area-under curve (AUC), above baseline, for ISR, glucose, insulin and C-peptide were calculated using the trapezoidal rule for the first 10 min and the entire 240 min of the FSIGT are referred to as the first phase AUC ($ISR_{first\ phase}$) and the total AUC (ISR_{total}), respectively.

FE_L and CLp were calculated using a method previously described by Polidori *et al.* (29). Hepatic insulin clearance was described using both linear and saturable kinetics. The linear model estimated a single parameter for hepatic insulin clearance (FE_L), while the saturable model estimated two parameters (Vmax and Km). The linear model described the hepatic insulin clearance well (fractional standard deviation <5%) in 91% (39/43) of participants. In addition, the linear model described the plasma insulin values with a mean normalized root square error (NMRSE) ±S.D. of 8.3 ± 2.14%. The parameters of the models were estimated using WinSAAM (30).

Statistical analysis

Data were analysed using STATA 12.0 (College Station, TX, USA). Data analysis included those that completed an

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FSIGT (n= 43). The cohort was divided into tertiles based on the DI, calculated as S_I × AIRg: DI_{Low}, $DI_{Intermediate}$ and DI_{High}. Normally and non-normally distributed data were expressed as mean ± S.D. and median (25–75th percentile), respectively. Differences between DI tertiles were determined using one-way ANOVA with a Bonferroni *post hoc test* or Kruskal–Wallis test with the Dunn *post hoc* test for normally and non-normally distributed data, respectively. An exploratory analysis, to determine the associations between DI and its components and central and ectopic fat depots, was conducted using Pearson (normally distributed variables) and Spearman (non-normally distributed variables) correlations and, therefore, no corrections for multiple correlations were performed. Linear regression was performed with the transformed variables to determine the association between DI components and central fat depots. Since S_I may confound the associations of AIRg, ISR and FE_L with central fat depots, we adjusted for S_I.

Results

Subject characteristics of the overall sample and by DI tertiles

A hyperbolic relationship between S_I and AIRg, the main components of DI, is presented in Fig. 1 for the overall sample (A) and in each of the DI tertiles (B). The cohort had a median age of 23 (IQR 21–27) years and a mean BMI of 33.2 ± 2.8 kg/m^2. Participant characteristics by DI tertile, as an estimate of β-cell function, are displayed in Table 1. The group with the highest DI were the most insulin sensitive (*post hoc* P=0.001) and had the highest glucose effectiveness (*post hoc* P < 0.001) compared to the group with the lowest DI. AIRg and $ISR_{first\ phase}$ showed no significant decline across DI tertiles (*post hoc* P = 0.060 and P = 0.071, respectively). In addition, the group with the highest DI had the highest CLp (*post hoc* P = 0.001) and lowest FE_L (*post hoc* P = 0.049) compared to those in the lowest DI tertile. Further, the highest DI had the lowest pancreatic and hepatic fat, and the lowest VAT and VAT-aSAT ratio, compared to those with the lowest DI.

Correlations of DI with AIRg, S_I and ISR

DI was positively associated with both its immediate components (AIRg: rho 0.372, P=0.014; S_I: rho 0.459, P = 0.020) and $ISR_{first\ phase}$ (rho 0.350, P = 0.021).

Associations of DI and its components with CLp and FE_L

Further, in univariate analysis, DI was positively associated with CLp and inversely with FE_L which explained 23.4 and 16.8% of the variance in DI, respectively (Table 2). However, when both CLp and FE_L were placed in the model only CLp remained a significantly positive correlate of DI. S_I was not associated with FE_L (P=0.065) and CLp (P=0.881) in the univariate model (Table 2). The association between S_I and FE_L became significant when adjusted for CLp (P=0.038). AIRg was associated with CLp in the univariate analysis but after adjusting for FE_L and S_I this association was diminished (Table 2).

Contributions of FE_L and $ISR_{first\ phase}$ to AIRg

In addition, we used linear regression models to determine the relative contributions of FE_L and $ISR_{first\ phase}$ to AIRg, without and with adjustment for S_I (Table 3). In the univariate models, $ISR_{first\ phase}$ explained more of the variance in AIRg compared to FE_L, 81.9% and 57.6%, respectively. S_I explained 32% of the variance in AIRg (data not shown). In the multivariate model, FE_L and $ISR_{first\ phase}$ were independently associated with AIRg, adjusted for S_I.

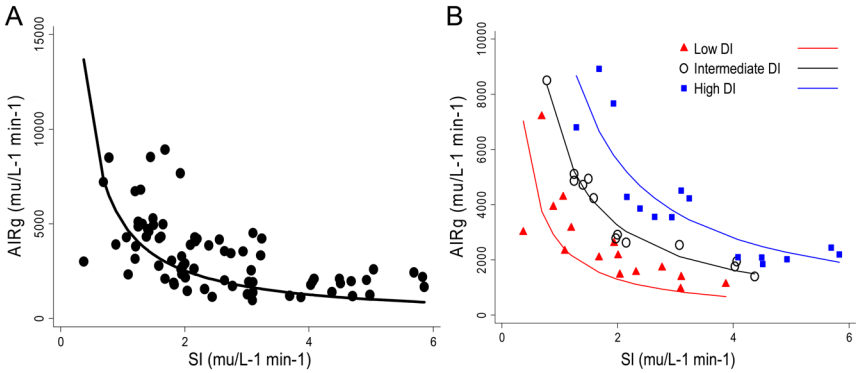

Figure 1
Hyperbolic association between insulin sensitivity (S_I) and acute insulin response to glucose (AIRg) for overall sample (A) and by disposition index (DI) tertiles (B).

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Table 1 Characteristics of participants by disposition index tertile.

	DI_{Low} (≤5073.4), *n* = 15	$DI_{Intermediate}$ (>5073.4 – ≤7933.2), *n* = 13	DI_{High} (>7933.2), *n* = 15	*P* value
		Mean ± s.d./Median (IQR)		
Age and anthropometry				
Age (years)	24 (21–28)	22 (21–28)	23 (21–24)	0.455
Body weight (kg)	85.1 ± 8.8	87.8 ± 6.3	87.1 ± 13.7	0.400
BMI (kg/m^2)	33.3 ± 2.6	35.3 ± 2.1	33.6 ± 3.5	0.147
Waist circumference (cm)	103.1 ± 5.3	103.9 ± 8.5	105.8 ± 11.0	0.705
Waist-hip ratio	0.89 (0.84–0.95)	0.89 (0.87–0.91)	0.90 (0.85–0.94)	0.899
Body composition and fat distribution				
Body fat mass (%)	50.1 ± 2.8	51.3 ± 3.1	50.4 ± 4.5	0.660
Fat free soft tissue mass (kg)	36.9 (33.8–40.3)	37.4 (35.8–38.8)	38.7 (34.9–40.6)	0.791
Trunk fat mass (%)	47.9 ± 4.7	47.3 ± 4.0	46.5 ± 4.7	0.684
Leg fat mass (%)	40.0 ± 5.2	40.6 ± 4.3	40.8 ± 5.2	0.901
Android fat mass (%)	8.5 ± 1.4	8.1 ± 0.8	7.9 ± 0.8	0.284
Gynoid fat mass (%)	18.5 ± 1.8	18.7 ± 2.0	18.4 ± 2.5	0.942
VAT (cm^3)	1113.3 ± 317.9	931.6 ± 355.1	669.8 ± 229.1[†]	0.002
aSAT (cm^3)	5303.9 ± 1116.8	5741.4 ± 1105.2	5629.1 ± 2191.8	0.747
VAT-aSAT	0.20 (0.16–0.24)	0.15 (0.11–0.21)[§]	0.13 (0.09–0.15)[†]	0.003
Ectopic fat				
Mean pancreatic fat (%)	8.0 (7.3–9.0)	7.4 (6.6–10.4)	6.2 (5.2–6.4)[†,‡]	0.006
Hepatic fat (%)	5.6 (4.5–6.1)	5.9 (4.5–8.4)	4.5 (3.9–4.9)[†,‡]	0.023
Soleus fat (%)	10.7 (10.1–11.4)	9.7 (8.8–11.6)	9.4 (6.8–10.3)	0.111
Tibialis anterior fat (%)	4.1 (3.3–5.7)	4.2 (3.5–5.4)	5.0 (2.6–6.1)	0.953
SIMCL (%)	2.9 (2.5–3.9)	2.9 (2.5–4.8)	2.1 (1.3–3.1)[†]	0.055
SEMCL (%)	4.5 (4.2–12.8)	5.1 (3.6–4.5)	4.3 (3.6–5.6)	0.410
TIMCL (%)	0.5 (0.3–0.8)	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.288
TEMCL (%)	2.9 (1.1–4.7)	2.6 (1.6–3.0)	2.5 (1.5–4.2)	0.815
Fasting metabolic parameters				
Fasting glucose (mmol/L)	5.4 ± 0.9	5.0 ± 0.9	5.2 ± 0.6	0.411
Fasting insulin (pmol/L)	85.2 (63.9–119.1)	82.5 (78–113.7)	57.7 (38.6–88.5)	0.107
Fasting C-peptide (pmol/L)	605.7 (572.6–744.8)	733.2 (491.5–835.8)	466.7 (382.3–787.8)	0.218
C-peptide/insulin$_{basal}$	6.8 (6.3–8.4)	8.4 (6.4–9.3)	9.0 (7.7–10.9)	0.087
ISR$_{basal}$ (pmol/min)	78.7 (74.4–96.8)	94.7 (62.9–102.4)	62.4 (49.7–104.6)	0.567
HOMA2 IR%	2.1 (1.7–2.9)	2.0 (1.8–2.7)	1.4 (1.0–2.2)	0.149
HOMA2 B%	134.6 (97.5–204.3)	182.6 (130.4–213.2)	108.7 (85.2–149.1)	0.133
HbA1c and FSIGT-derived measures				
HbA1c	5.24 ± 0.36	5.19 ± 0.36	5.20 ± 0.43	0.829
Disposition index (AU)	3780 (2989–4527)	6596 (6070–7119)[§]	9995 (9247–13882)[†,‡]	<0.001
S_I (mU/L/min)	1.94 (1.1–2.8)	1.97 (1.4–3.1)	3.1 (2.2–4.5)[†,‡]	0.008
AIR_g (mU/L/min)	2159 (1465–3159)	2909 (2544–4870)	3561 (4278–4514)	0.064
Sg (%/min)	0.017 ± 0.008	0.024 ± 0.008	0.031 ± 0.008[†]	<0.001
ISR$_{first\ phase}$ (pmol/min)	2692 (1909–4023)	3624 (2826–4999)	4049 (2758–4877)	0.060
ISR$_{total}$ (pmol/min)	4246 (3231–8095)	5589 (4813–7913)	4272 (3261–6500)	0.365
FE_L (%)	61.6 ± 11.9	54.5 ± 8.7	51.4 ± 9.4[†]	0.042
CLp (mL/min/kg)	74.7 (61.3–105.3)	91.5 (77.1–125.8)	128.9 (92.3–168.2)[‡]	0.009

Mean (±s.d.) for normally distributed variables and median (IQR) for non-normally distributed variables.
P-value derived from ANOVA or Kruskal–Wallis, significant *post hoc* differences: [†]High DI compared to low DI; [‡]High DI compared to intermediate DI; [§]Intermediate DI compared to low DI with $P \leq 0.05$ deemed as statistically significant.
AIRg, acute insulin response to glucose; aSAT, abdominal s.c. adipose tissue; CLp, peripheral insulin clearance; DI, disposition index; FEL, fractional hepatic insulin extraction; HOMA, homeostatic model assessment 2 of insulin resistance (IR) and beta cell function (B); ISR, insulin secretion rate; SEMCL, soleus extra-myocellular fat; Sg, glucose effectiveness; SI, insulin sensitivity; SIMCL, soleus intra-myocellular fat; TEMCL, tibialis anterior extra-myocellular fat; TIMCL, tibialis anterior intra-myocellular fat; VAT, visceral adipose tissue. adipose tissue; SIMCL, soleus intra-myocellular fat; SEMCL, soleus extra-myocellular fat; TIMCL, tibialis anterior intra-myocellular fat; TEMCL, tibialis anterior extra-myocellular fat; ISR, insulin secretion rate; HOMA, homeostatic model assessment 2 of insulin resistance (IR) and beta cell function (B); SI, insulin sensitivity; AIRg, acute insulin response to glucose; Sg, glucose effectiveness; FE

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Table 2 Relationship of β-cell function and insulin sensitivity with hepatic and peripheral insulin clearance.

Model	Variables	Sqrt DI		Ln AIR_g		ln $ISR_{first\ phase}$		ln S_I	
		β (*P* value)	R^2	β (*P* value)	R^2	β (*P* value)	R^2	β (*P* value)	R^2
1.	FE_L (%)	−0.709 (0.010)	0.168	−0.016 (<0.001)	0.576	−0.011 (<0.001)	0.403	0.008 (0.065)	0.089
2.	ln CLp (mL/min/kg)	55.80 (0.002)	0.234	0.623 (0.004)	0.204	0.644 (<0.001)	0.293	−0.042 (0.881)	0.001
3.	FE_L (%)	−0.364 (0.222)	0.265	−0.015 (<0.001)	0.579	−0.009 (0.002)	0.430	0.010 (0.038)	0.115
	ln CLp (mL/min/kg)	42.78 (0.035)		0.086 (0.630)		0.335 (0.060)		0.320 (0.313)	
4.	FE_L (%)	–		−0.011 (<0.001)	0.765	−0.005 (0.023)	0.647	–	
	ln CLp (mL/min/kg)	–		0.206 (0.138)		0.438 (0.004)		–	
	ln S_I ($(mU/L)^{-1}\ min^{-1}$)	–		−0.374 (<0.001)		−0.324 (<0.001)		–	

$P \leq 0.05$ regarded as statistically significant.
AIRg, acute insulin response to glucose; CLp, peripheral insulin clearance; DI, disposition index; FE_L, fractional hepatic insulin extraction; ISR, insulin secretion rate; Ln, natural log; S_I, insulin sensitivity; Sqrt, square root; β, linear regression coefficient.

DI and its components – associations with body fat distribution and ectopic fat

DI was not associated with fat mass (%), leg fat mass or aSAT (data not shown) but was negatively associated with VAT and the VAT-aSAT ratio (β −167.4, $P < 0.001$). The unadjusted associations between DI and ectopic fat depots and VAT are shown in Fig. 2. DI was inversely correlated with pancreatic fat, total soleus fat and both soleus IMCL and EMCL. DI was not associated with tibialis anterior fat (β −2.59, $P=0.887$). In univariate analysis, VAT explained 32% of the variance in DI, while soleus IMCL explained 18.7%, soleus EMCL 10.9% and pancreatic fat 12%. Adjusting for age and fat mass (%) in multivariable models did not alter the models. Accordingly, the negative associations of DI with pancreatic fat (β −62.9, $P=0.030$), VAT (β −0.037, $P < 0.001$), soleus fat (β −50.0, $P = 0.049$), soleus IMCL (β −41.3, $P = 0.012$) and soleus EMCL (β −32.0, $P = 0.047$) remained. We explored the associations between DI and ectopic fat, independent of VAT and found that the associations of DI with pancreatic fat, hepatic fat, soleus fat and soleus IMCL were ameliorated to non-significance ($P > 0.05$) (data not shown). Instead, VAT remained a significant determinant of DI independent of any of the ectopic fat depots (data not shown).

The components of DI (S_I, AIRg, $ISR_{first\ phase}$, FE_L and CLp) were not associated with fat mass (%) or ectopic fat sites ($P > 0.05$) (data not shown). Rather, significant associations were found between the components of DI and central fat measures (Fig. 3 and Supplementary Table 1, see section on supplementary materials given at the end of this article). VAT was associated with lower S_I and AIRg, as well as a lower $ISR_{first\ phase}$ and higher FE_L. CLp was not associated with any central fat depots (data not shown).

Lastly, we explored the associations between VAT and ectopic fat depots (Fig. 3 and Supplementary Table 2). VAT was positively associated with pancreatic fat (rho 0.417, $P = 0.007$) and soleus IMCL (rho 0.403, $P = 0.014$) but was not associated with soleus EMCL (rho 0.121, $P=0.474$) and hepatic fat (rho 0.300, $P=0.060$). Moreover, pancreatic fat was positively associated with hepatic fat (rho 0.540, $P < 0.001$).

Discussion

The correlates of β-cell function, a critical factor in the pathogenesis of T2D, are incompletely understood, especially in black African populations who present with a phenotype of low S_I, hyperinsulinaemia and low ectopic fat deposition. Our study extends the existing evidence

Table 3 The contribution of insulin secretion and hepatic insulin extraction to acute insulin response to glucose.

Model	Variables	Ln AIR_g			
		β-coefficient ± S.E.	Partial beta	*P*	Model R^2
1.	ln $ISR_{first\ phase}$	0.99 ± 0.07	–	<0.001	0.819
2.	Hepatic insulin extraction (%)	−0.016 ± 0.002	–	<0.001	0.576
3.	ln $ISR_{first\ phase}$	0.813 ± 0.091	0.701	<0.001	0.869
	Hepatic insulin extraction (%)	−0.007 ± 0.002	−0.314	0.001	
4.	ln $ISR_{first\ phase}$	0.667 ± 0.094	0.574	<0.001	0.898
	Hepatic insulin extraction (%)	−0.007 ± 0.001	−0.333	<0.001	
	ln S_I	−0.166 ± 0.053	−0.203	0.004	

AIRg, acute insulin response to glucose; ISR, insulin secretion rate; Ln, natural log; S.E., standard error; S_I, insulin sensitivity.

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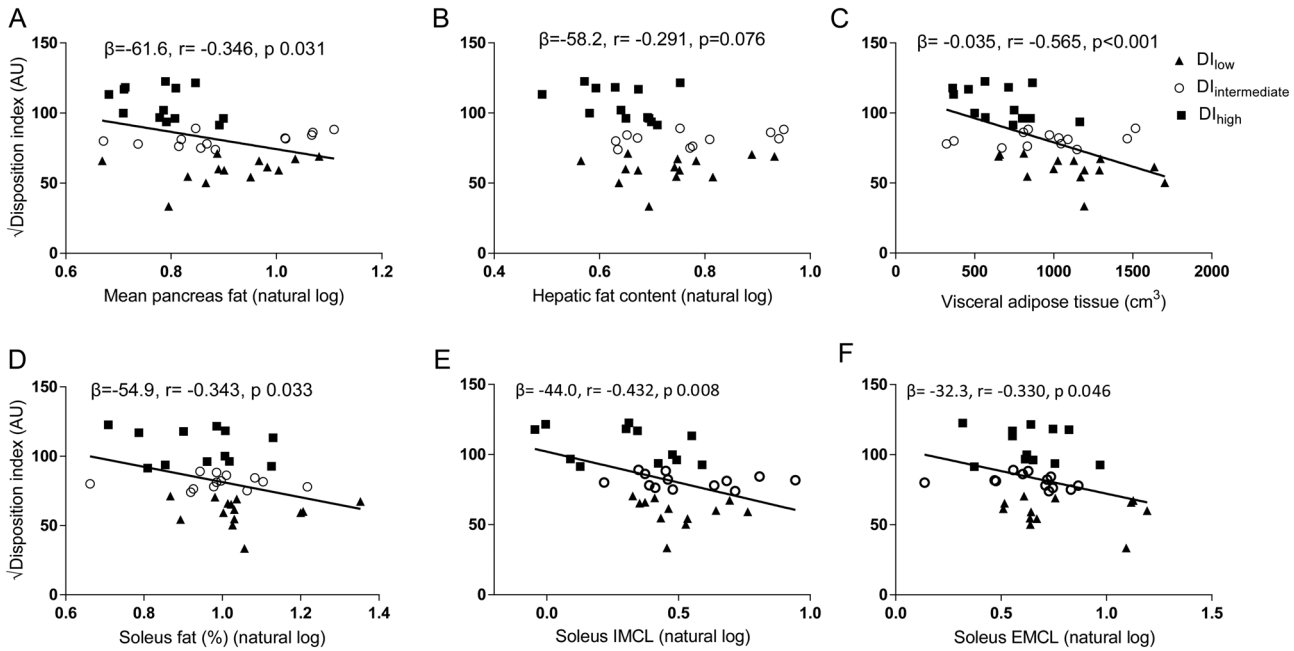

Figure 2
Disposition index (DI) associations with (A) pancreatic fat (B) hepatic fat, (C) visceral adipose tissue, (D) soleus fat (E) soleus intra-myocellar fat (IMCL) and (F) soleus extra-myocellular fat (EMCL) in those with low DI (solid black triangles), intermediate DI (open circles) and high DI (solid black square). Regression line indicates significant association ($P < 0.05$) in the whole cohort, β (slope), r – correlation coefficients.

by demonstrating that DI was positively associated with CLp. Notably, the major correlate of AIRg was $ISR_{first\ phase}$, above insulin clearance. VAT emerged as the strongest correlate of DI, above and independent of pancreatic fat and soleus fat. Additionally, we showed that a higher VAT was also associated with a lower $ISR_{first\ phase}$ and higher FE_L. Thus, our findings suggest that VAT is a more important correlate of a lower DI than ectopic fat in this cohort, not only through its association with a lower S_I but also through its relation with the AIRg downstream components, ISR and FE_L (Fig. 3).

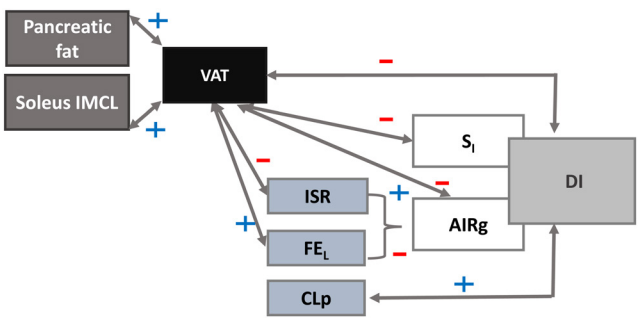

Figure 3
Graphical summary of the important correlates of disposition index (DI) shown in the present study. A higher DI was associated with a lower pancreatic and soleus intra-myocellular fat (IMCL), but these associations were not independent from VAT. A higher VAT was associated with lower first phase insulin secretion rate (ISR) and higher hepatic insulin extraction (FE_L). Accordingly, VAT is a more important correlate of a lower DI than ectopic fat in this cohort, not only through its association with a lower insulin sensitivity (S_I) but also through its relationship with the acute insulin response (AIRg) and its downstream components, ISR and FE_L. Peripheral insulin clearance (CLp) was associated with DI and not S_I. Positive association (+); negative association (–).

DI associates with peripheral insulin clearance

Our study not only distinguishes between FE_L and CLp, which has only been done in a few studies in adults without T2D (4, 29, 31), but we also demonstrated a positive association between DI and CLp for the first time(Fig. 3). The reason for this relationship is unclear. Nevertheless, considering DI is based on the product of S_I and AIRg, we can postulate two scenarios. First, a higher DI may be due to hyperinsulinaemia relative to the level of S_I. However, hyperinsulinaemia has been associated with lower insulin clearance in both adipose tissue (32) and muscle (33), due to reduced affinity of insulin receptors at these sites and is, therefore, an unlikely explanation for our finding of a higher CLp relative to higher DI. Secondly, a higher DI could be due to a greater S_I in relation to the level of AIRg. In this scenario, a higher CLp may be due to enhanced binding of insulin to insulin receptors in peripheral tissues. Although a positive association has been demonstrated between S_I and

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hepatic insulin clearance (34), no association between insulin internalization, a measure of insulin clearance, and S_I was observed in rat adipocytes (32), whereas the association between S_I and insulin clearance in muscle is unknown. However, in support of the second scenario, we showed that those with the highest DI and CLp were also more insulin sensitive but only exhibited a slightly higher AIRg compared to those with lower DI and CLp. Nevertheless, CLp was not associated with S_I in our study but rather with the insulin secretory component. Further, we may also consider that the observed association between DI and CLp is in compensation for a lower FE_L, but this association remained independent of FE_L. We also evaluated the associations of CLp with ectopic fat deposition as possible explanation for the association with DI but found that CLp was not associated with skeletal muscle fat. Skeletal muscle fat has been associated with reduced S_I (35), which may also affect CLp in the muscle. Further study is justified to evaluate the relationship between CLp and S_I in the muscle, considering that the muscle is only secondary to the kidney in the proportion of insulin cleared in the periphery (36). Of note, CLp in our study is much higher compared to another study conducted in black American women (4). However, the acute insulin response in our cohort was twice as high as that observed in Piccinini *et al.* and may contribute to this discrepancy.

Associations between DI and ectopic fat

Preservation of β-cell function is critical for delaying the onset of T2D. We, therefore, investigated, first, whether ectopic fat accumulation may explain the variance in DI and secondly, we assessed the associations of ectopic fat with the components of DI. Our study demonstrated that a higher DI was associated with a lower pancreatic and soleus fat but these associations were not independent of VAT. A similar observation was found in overweight African–American and Hispanic adolescents (13 to 25 years old) without T2D (37). In contrast, a positive association between DI and pancreatic fat, adjusted for BMI and VAT, was shown in black African–American women (11). However, this study included participants with and without T2D, which may explain the incongruent findings. Our study extends the literature by showing that pancreatic fat was not associated with $ISR_{first\ phase}$ or with FE_L in black Africans. Notably, a positive association was found between pancreatic fat and VAT in our study (Fig. 3). Our findings, therefore, suggest that pancreatic fat may only be a marker of VAT accumulation and may not be detrimental to β-cell function in this cohort.

Contribution of hepatic insulin clearance and ISR to AIRg

The ability to maintain DI and prevent deteriorating glucose tolerance depends on the balance between AIRg and S_I (1). However, there is no consensus on the mechanism of maintaining a higher AIRg, which is frequently observed in black African populations (2, 3). Some studies reported that a lower hepatic insulin clearance alone is responsible for a higher AIRg (38, 39), while others showed that both lower hepatic insulin clearance and higher ISR contribute towards a higher AIRg (3, 4). Notably, AIRg may be out of proportion for the level of S_I and to assess the relative contribution of $ISR_{first\ phase}$ and insulin clearance to AIRg in this context, we need to adjust for S_I. Accordingly, a lower FE_L and higher $ISR_{first\ phase}$ were the main independent contributors towards a higher AIRg, but $ISR_{first\ phase}$ explained more of the variance in AIRg. Lowering hepatic insulin clearance is an important compensatory mechanism to reduce the strain on the pancreatic β-cells, which has been shown in canines (40). Indeed, we also noted a higher DI is associated with a lower FE_L which may be explained by the negative association between AIRg and FE_L (Fig. 3). Further, we showed that higher ISR associates with AIRg, independently of lower FE_L and S_I, suggesting that despite a lower FE_L, pancreatic β-cells may continue to secrete insulin at a higher rate, which may be detrimental to the longevity of the β-cell in this cohort.

Associations of ISR and hepatic insulin clearance with ectopic fat

The current study further examined the associations between the components of AIRg and ectopic fat. Previously, reduced hepatic insulin clearance and increased insulin secretion have been associated with hepatic fat accumulation (41). An association between fasting hepatic insulin clearance and hepatic fat was studied in African–American women (13), but the association between stimulated hepatic insulin clearance, which is a more physiological response, and hepatic fat has not been previously investigated in black African populations without T2D. We showed that hepatic fat was not associated with FE_L and ISR in black South African women with obesity. Therefore, in black African populations, hepatic fat may not be an important correlate

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of hepatic insulin clearance and insulin secretion prior to T2D, and also not in those with early T2D (42). Instead, we found that a lower $ISR_{first\ phase}$ and higher FE_L were associated with higher VAT (Fig. 3). This highlights a novel mechanism to explain the association between AIRg and VAT since to the best of our knowledge, no previous study has evaluated the effect of central fat depots on FE_L. In addition, evidence on the associations between central fat depots and ISR determined by C-peptide deconvolution is also limited in black African populations (9). We add to the literature by showing a negative association between VAT and $ISR_{first\ phase}$, independent of S_I, in premenopausal black South African women. Interestingly, black African women have lower VAT compared to other ethnicities (2, 3), but they have a greater propensity to increase VAT over time, with the greatest increase occurring in the 20–29-year age group (43). However, further studies are needed to confirm the effect of VAT on ISR in this cohort.

Associations between S_I and ectopic fat

We also assessed the association between S_I and ectopic fat and found no association between S_I and skeletal muscle or hepatic fat. These findings suggest that these ectopic fat depots are not important correlates of S_I in our study. However, we should consider that we measured whole body S_I, which could have diluted the associations with hepatic and soleus fat. Indeed, a small South African study found that in black South African women, hepatic fat was associated with hepatic S_I but not peripheral S_I (12). Furthermore, total soleus fat and soleus IMCL were associated with peripheral S_I but not hepatic S_I (12). Nevertheless, lower S_I was associated with higher VAT which may contribute to the observed inverse association between DI and VAT in this cohort (Fig. 3).

A major strength of this study is that ISR and both hepatic and peripheral clearance were determined using mathematical modelling, which has never been done before in an African cohort. Further, we assessed pancreatic, hepatic and skeletal muscle ectopic fat depots, as well as VAT and aSAT volumes using MRI. However, this study also has limitations. While we cannot exclude that only progesterone injectable contraception usage may have contributed to the high AIRg observed in this cohort (44), it is important to note that a high AIRg has been reported in African–American children prior to the use of contraception (5, 45) and in many other studies including African women in the USA (3, 46, 47) and SA (2). We did not determine glucose tolerance, and we did not distinguish between hepatic and peripheral S_I. We have not corrected for multiple correlations which may have produced false positive associations. However, the focus was to explore the possible significant associations between variables and, therefore, we wanted to minimize false negative associations. Of note, the sample size was modest and may have contributed to a type II error. In addition, these findings are applicable to premenopausal, black South African women with obesity and may not be extrapolated to men or other races. Lastly, a causal relationship between variables cannot be determined from this cross-sectional study and further research is required to confirm these exploratory findings.

In conclusion, an original finding from this study was that DI was associated with CLp, independent from FE_L. Further, while both FE_L and $ISR_{first\ phase}$ independently contributed towards hyperinsulinaemia, $ISR_{first\ phase}$ was more important. Ectopic fat was not an important independent correlate of DI and its components. Rather, a key finding was that higher VAT was the principal correlate of a lower DI, above other ectopic fat depots. Additionally, the associations of higher VAT on the downstream components of DI, a lower $ISR_{first\ phase}$ and higher FE_L may contribute to lower DI prior to T2D onset but requires further elucidation. Accordingly, the prevention of VAT accumulation, especially in young black African women should be an important target for β-cell preservation.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-21-0153.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

A M, J G, T O conceived and designed the study. M F, A M, J G, J S performed study procedures. M F, A A, J H, O H performed MRI fat quantification. M F, D S performed mathematical modelling. MF prepared figures and drafted

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the manuscript. M F, A M, J G were involved in data analysis. M F, A M, J G, T O, J S, S K, J H, D S, L G, A A, O H read and edited the manuscript and approved the final version. M F, A M and J G are the guarantors of this work and, as such, have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1 Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC & Palmer JP. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 1993 **42** 1663–1672. (https://doi.org/10.2337/diab.42.11.1663)

2 Goedecke JH, Dave JA, Faulenbach MV, Utzschneider KM, Lambert EV, West S, Collins M, Olsson T, Walker BR, Seckl JR, et al. Insulin response in relation to insulin sensitivity: an appropriate beta-cell response in black South African women. *Diabetes Care* 2009 **32** 860–865. (https://doi.org/10.2337/dc08-2048)

3 Chow CC, Periwal V, Csako G, Ricks M, Courville AB, Miller BV, Vega GL & Sumner AE. Higher acute insulin response to glucose may determine greater free fatty acid clearance in African-American women. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 2456–2463. (https://doi.org/10.1210/jc.2011-0532)

4 Piccinini F, Polidori DC, Gower BA & Bergman RN. Hepatic but not extrahepatic insulin clearance is lower in African-American than in European American women. *Diabetes* 2017 **66** 2564–2570. (https://doi.org/10.2337/db17-0413)

5 Gower BA, Granger WM, Franklin F, Shewchuk RM & Goran MI. Contribution of insulin secretion and clearance to glucose-induced insulin concentration in African-American and Caucasian children. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 2218–2224. (https://doi.org/10.1210/jcem.87.5.8498)

6 Toffolo G, Campioni M, Basu R, Rizza RA & Cobelli C. A minimal model of insulin secretion and kinetics to assess hepatic insulin extraction. *American Journal of Physiology: Endocrinology and Metabolism* 2006 **290** E169–E176. (https://doi.org/10.1152/ajpendo.00473.2004)

7 Sumner AE, Farmer NM, Cochran CS, Seeking NG, Vanevski K, Reynolds JC, Premkumar A & Boston RC. Obese premenopausal African-American women with normal and impaired glucose tolerance have a similar degree of insulin resistance but differ in β-cell function. *Diabetes Care* 2001 **24** 1978–1983. (https://doi.org/10.2337/diacare.24.11.1978)

8 Wagenknecht LE, Langefeld CD, Scherzinger AL, Norris JM, Haffner SM, Saad MF & Bergman RN. Insulin sensitivity, insulin secretion, and abdominal fat: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes* 2003 **52** 2490–2496. (https://doi.org/10.2337/diabetes.52.10.2490)

9 Chandler-Laney PC, Phadke RP, Granger WM, Mũoz JA, Man CD, Cobelli C, Ovalle F, Fernandz JR & Gower BA. Adiposity and B-cell function: relationships differ with ethnicity and age. *Obesity* 2010 **18** 2086–2092. (https://doi.org/10.1038/oby.2010.44)

10 Szczepaniak LS, Victor RG, Mathur R, Nelson MD, Szczepaniak EW, Tyer N, Chen I, Unger RH, Bergman RN & Lingvay I. Pancreatic steatosis and its relationship to β-cell dysfunction in humans: racial and ethnic variations. *Diabetes Care* 2012 **35** 2377–2383. (https://doi.org/10.2337/dc12-0701)

11 Lingvay I. Ethnic diversity in beta-cell function susceptibility to pancreatic triglyceride levels: pilot investigation. *Journal of Diabetes and Metabolism* 2014 **5** 3. (https://doi.org/10.4172/2155-6156.1000348)

12 Goedecke JH, Keswell D, Weinreich C, Fan J, Hauksson J, Victor H, Utzschneider K, Levitt NS, Lambert EV, Kahn SE, et al. Ethnic differences in hepatic and systemic insulin sensitivity and their associated determinants in obese black and white South African women. *Diabetologia* 2015 **58** 2647–2652. (https://doi.org/10.1007/s00125-015-3720-7)

13 Chung ST, La Cruz MG De, Aldana PC, Mabundo LS, DuBose CW, Onuzuruike AU, Walter M, Gharib AM, Courville AB, Sherman AS, et al. Postprandial insulin response and clearance among black and white women: the federal women's study. *Journal of Clinical Endocrinology and Metabolism* 2019 **104** 181–192. (https://doi.org/10.1210/jc.2018-01032)

14 Goedecke JH, Mendham AE, Clamp L, Nono Nankam PA, Fortuin-De Smidt MC, Phiri L, Micklesfield LK, Keswell D, Woudberg NJ, Lecour S, et al. An exercise intervention to unravel the mechanisms underlying insulin resistance in a cohort of black South African women: protocol for a randomized controlled trial and baseline characteristics of participants. *Journal of Medical Internet Research* 2018 **20** e75. (https://doi.org/10.2196/resprot.9098)

15 Berenson AB, Van Den Berg P, Williams KJ & Rahman M. Effect of injectable and oral contraceptives on glucose and insulin levels. *Obstetrics and Gynecology* 2011 **117** 41–47. (https://doi.org/10.1097/AOG.0b013e318202ac23)

16 Cooper D & Marks AS. Community-based distribution of contraception: a pilot project in Khayelitsha, Cape Town. *Urbanisation and Health Newsletter* 1996 **30** 49–55.

17 Goedecke JH, Micklesfield LK, Levitt NS, Lambert EV, West S, Maartens G & Dave JA. Effect of different antiretroviral drug regimens on body fat distribution of HIV-infected South African women. *AIDS Research and Human Retroviruses* 2013 **29** 557–563. (https://doi.org/10.1089/aid.2012.0252)

18 Machann J, Bachmann OP, Brechtel K, Dahl DB, Wietek B, Klumpp B, Häring HU, Claussen CD, Jacob S & Schick F. Lipid content in the musculature of the lower leg assessed by fat selective MRI: intra- and inter-individual differences and correlation with anthropometric and metabolic data. *Journal of Magnetic Resonance Imaging* 2003 **17** 350–357. (https://doi.org/10.1002/jmri.10255)

19 Hakim O, Bonadonna RC, Mohandas C, Billoo Z, Sunderland A, Boselli L, Alberti KGMM, Peacock JL, Umpleby AM, Edwards GC, et al. Associations between pancreatic lipids and β-cell function in black african and white European men with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* 2019 **104** 1201–1210. (https://doi.org/10.1210/jc.2018-01809)

20 Shen W, Chen J, Gantz M, Velasquez G, Punyanitya M & Heymsfield SB. A single MRI slice does not accurately predict visceral and subcutaneous adipose tissue changes during weight loss. *Obesity* 2012 **20** 2458–2463. (https://doi.org/10.1038/oby.2012.168)

21 Hu F, Yang R, Huang Z, Wang M, Yuan F, Xia C, Wei Y & Song B. 3D multi-echo dixon technique for simultaneous assessment of liver steatosis and iron overload in patients with chronic liver diseases: a feasibility study. *Quantitative Imaging in Medicine and Surgery* 2019 **9** 1014–1024. (https://doi.org/10.21037/qims.2019.05.20)

22 Heber SD, Hetterich H, Lorbeer R, Bayerl C, Machann J, Auweter S, Storz C, Schlett CL, Nikolaou K, Reiser M, et al. Pancreatic fat content by magnetic resonance imaging in subjects with prediabetes, diabetes, and controls from a general population without cardiovascular disease. *PLoS ONE* 2017 **12** e0177154. (https://doi.org/10.1371/journal.pone.0177154)

23 Alizai H, Nardo L, Karampinos DC, Joseph GB, Yap SP, Baum T, Krug R, Majumdar S & Link TM. Comparison of clinical semi-quantitative assessment of muscle fat infiltration with quantitative

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assessment using chemical shift-based water/fat separation in MR studies of the calf of post-menopausal women. *European Radiology* 2012 **22** 1592–1600. (https://doi.org/10.1007/s00330-012-2404-7)
24 Bonekamp S, Ghosh P, Crawford S, Solga SF, Horska A, Brancati FL, Diehl AM, Smith S & Clark JM. Quantitative comparison and evaluation of software packages for assessment of abdominal adipose tissue distribution by magnetic resonance imaging. *International Journal of Obesity* 2008 **32** 100–111. (https://doi.org/10.1038/sj.ijo.0803696)
25 Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM & Bergman RN. MINMOD millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technology and Therapeutics* 2003 **5** 1003–1015. (https://doi.org/10.1089/152091503322641060)
26 Korytkowski MT, Berga SL & Horwitz MJ. Comparison of the minimal model and the hyperglycemic clamp for measuring insulin sensitivity and acute insulin response to glucose. *Metabolism: Clinical and Experimental* 1995 **44** 1121–1125. (https://doi.org/10.1016/0026-0495(95)90003-9)
27 Bergman RN, Prager R, Volund A & Olefsky JM. Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *Journal of Clinical Investigation* 1987 **79** 790–800. (https://doi.org/10.1172/JCI112886)
28 Van Cauter E, Mestrez F, Sturis J & Polonsky KS. Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 1992 **41** 368–377. (https://doi.org/10.2337/diab.41.3.368)
29 Polidori DC, Bergman RN, Chung ST & Sumner AE. Hepatic and extrahepatic insulin clearance are differentially regulated: results from a novel model-based analysis of intravenous glucose tolerance data. *Diabetes* 2016 **65** 1556–1564. (https://doi.org/10.2337/db15-1373)
30 Stefanovski D, Moate PJ & Boston RC. WinSAAM: a windows-based compartmental modeling system. *Metabolism: Clinical and Experimental* 2003 **52** 1153–1166. (https://doi.org/10.1016/S0026-0495(03)00144-6)
31 Utzschneider KM, Kahn SE & Polidori DC. Hepatic insulin extraction in NAFLD is related to insulin resistance rather than liver fat content. *Journal of Clinical Endocrinology and Metabolism* 2019 **104** 1855–1865. (https://doi.org/10.1210/jc.2018-01808)
32 Jochen A, Hays J & Lee M. Kinetics of insulin internalization and processing in adipocytes: effects of insulin concentration. *Journal of Cellular Physiology* 1989 **141** 527–534. (https://doi.org/10.1002/jcp.1041410311)
33 Eggleston EM, Jahn LA & Barrett EJ. Hyperinsulinemia rapidly increases human muscle microvascular perfusion but fails to increase muscle insulin clearance: evidence that a saturable process mediates muscle insulin uptake. *Diabetes* 2007 **56** 2958–2963. (https://doi.org/10.2337/db07-0670)
34 Jones CNO, Pei D, Staris P, Polonsky KS, Chen YDI & Reaven GM. Alterations in the glucose-stimulated insulin secretory dose-response curve and in insulin clearance in nondiabetic insulin-resistant individuals. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1834–1838. (https://doi.org/10.1210/jcem.82.6.3979)
35 Goodpaster BH, Thaete FL, Simoneau JA & Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes* 1997 **46** 1579–1585. (https://doi.org/10.2337/diacare.46.10.1579)
36 Tokarz VL, MacDonald PE & Klip A. The cell biology of systemic insulin function. *Journal of Cell Biology* 2018 **217** 2273–2289. (https://doi.org/10.1083/jcb.201802095)
37 Le KA, Ventura EE, Fisher JQ, Davis JN, Weigensberg MJ, Punyanitya M, Hu HH, Nayak KS & Goran MI. Ethnic differences in pancreatic fat accumulation and its relationship with other FatDepots and inflammatory markers. *Diabetes Care* 2011 **34** 485–490. (https://doi.org/10.2337/dc10-0760)
38 Osei K & Schuster DP. Ethnic differences in secretion, sensitivity, and hepatic extraction of insulin in black and white Americans. *Diabetic Medicine* 1994 **11** 755–762. (https://doi.org/10.1111/j.1464-5491.1994.tb00349.x)
39 Osei K, Schuster DP, Owusu SK & Amoah AGB. Race and ethnicity determine serum insulin and C-peptide concentrations and hepatic insulin extraction and insulin clearance: comparative studies of three populations of West African ancestry and white Americans. *Metabolism: Clinical and Experimental* 1997 **46** 53–58. (https://doi.org/10.1016/S0026-0495(97)90167-0)
40 Kim SP, Ellmerer M, Kirkman EL & Bergman RN. B-cell 'rest' accompanies reduced first-pass hepatic insulin extraction in the insulin-resistant, fat-fed canine model. *American Journal of Physiology: Endocrinology and Metabolism* 2007 **292** E1581–E1589. (https://doi.org/10.1152/ajpendo.00351.2006)
41 Bergman RN, Kim SP, Catalano KJ, Hsu IR, Chiu JD, Kabir M, Hucking K & Ader M. Why visceral fat is bad: mechanisms of the metabolic syndrome. *Obesity* 2006 **14** (Supplement 1) 16S–19S. (https://doi.org/10.1038/oby.2006.277)
42 Hakim O, Bello O, Bonadonna RC, Mohandas C, Shojaee-Moradie F, Jackson N, Boselli L, Whitcher B, Shuaib H, Alberti KGMM, et al. Ethnic differences in intrahepatic lipid and its association with hepatic insulin sensitivity and insulin clearance between men of black and white ethnicity with early type 2 diabetes. *Diabetes, Obesity and Metabolism* 2019 **21** 2163–2168. (https://doi.org/10.1111/dom.13771)
43 Hairston KG, Scherzinger A, Foy C, Hanley AJ, McCorkle O, Haffner S, Norris JM, Bryer-Ash M & Wagenknecht LE. Five-year change in visceral adipose tissue quantity in a minority cohort: the insulin resistance atherosclerosis study (IRAS) family study. *Diabetes Care* 2009 **32** 1553–1555. (https://doi.org/10.2337/dc09-0336)
44 Kahn HS, Curtis KM & Marchbanks PA. Effects of injectable or implantable progestin-only contraceptives on insulin-glucose metabolism and diabetes risk. *Diabetes Care* 2003 **26** 216–225. (https://doi.org/10.2337/diacare.26.1.216)
45 Piccinini F, Polidori DC, Gower BA, Fernandez JR & Bergman RN. Dissection of hepatic versus extra-hepatic insulin clearance: ethnic differences in childhood. *Diabetes, Obesity and Metabolism* 2018 **20** 2869–2875. (https://doi.org/10.1111/dom.13471)
46 Ellis AC, Alvarez JA, Granger WM, Ovalle F & Gower BA. Ethnic differences in glucose disposal, hepatic insulin sensitivity, and endogenous glucose production among African American and European American women. *Metabolism: Clinical and Experimental* 2012 **61** 634–640. (https://doi.org/10.1016/j.metabol.2011.09.011)
47 Goree LLT, Darnell BE, Oster RA, Brown MA & Gower BA. Associations of free fatty acids with insulin secretion and action among African-American and European-American girls and women. *Obesity* 2010 **18** 247–253. (https://doi.org/10.1038/oby.2009.248)

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