

Ethnic differences in hepatic and systemic insulin sensitivity and their associated determinants in obese black and white South African women

Julia H. Goedecke^{1,2} · Dheshnie Keswell² · Carsten Weinreich³ · Jia Fan⁴ ·
Jon Hauksson⁵ · Hendriena Victor² · Kristina Utzschneider⁶ · Naomi S. Levitt³ ·
Estelle V. Lambert² · Steven E. Kahn⁶ · Tommy Olsson^{7,8}

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Abstract

Aims/hypothesis There is evidence to suggest that ectopic fat deposition in liver and skeletal muscle may differ between black and white women resulting in organ-specific differences in insulin sensitivity. Accordingly, the aim of the study was to examine ethnic differences in hepatic and peripheral insulin sensitivity, and the association with hepatic and skeletal muscle lipid content, and skeletal muscle gene expression.

Methods In a cross-sectional study including 30 obese premenopausal black and white women, body composition (dual energy x-ray absorptiometry), liver fat and skeletal muscle (soleus and tibialis anterior) fat accumulation (proton-magnetic resonance spectroscopy), skeletal muscle gene expression, insulin sensitivity (two-step isotope labelled, hyperinsulinaemic–euglycaemic clamp with 10 mU m⁻² min⁻¹ and 40 mU m⁻² min⁻¹ insulin infusions), and serum adipokines were measured.

Results We found that, although whole-body insulin sensitivity was not different, obese white women presented

with lower hepatic insulin sensitivity than black women (% suppression of endogenous glucose production [% supp EGP], median [interquartile range (IQR)]: 17 [5–51] vs 56 [29–100] %, $p=0.002$). While liver fat tended to be lower ($p=0.065$) and skeletal muscle fat deposition tended to be higher ($p=0.074$) in black compared with white women, associations with insulin sensitivity were only observed in black women (% supp EGP vs liver fat: $r=-0.57$, $p<0.05$ and % supp EGP vs soleus fat: $r=-0.56$, $p<0.05$).

Conclusions/interpretation These findings may suggest that black women are more sensitive to the effects of ectopic lipid deposition than white women.

Keywords Black African · Ectopic fat · Ethnicity · Euglycaemic–hyperinsulinaemic clamp · Hepatic insulin sensitivity · Liver fat · Peripheral insulin sensitivity · Skeletal muscle lipid

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✉ Julia H. Goedecke
julia.goedecke@mrc.ac.za

¹ Non-Communicable Disease Research Unit, South African Medical Research Council, PO Box 19070, Tygerberg 7505, South Africa

² Division of Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town, Cape Town, South Africa

³ Division of Diabetes and Endocrinology, Department of Medicine, University of Cape Town, Cape Town, South Africa

⁴ MRC/UCT Medical Imaging Research Unit, Department of Human Biology, University of Cape Town, Cape Town, South Africa

⁵ Center for Medical Technology and Radiation Physics, Umeå University Hospital, Umeå, Sweden

⁶ Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, VA Puget Sound Health Care System and University of Washington, Seattle, WA, USA

⁷ Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

⁸ Stellenbosch Institute for Advanced Study (STIAS), Wallenberg Research Centre, Stellenbosch University, Stellenbosch, South Africa

Abbreviations

% supp EGP	% Suppression of endogenous glucose production
DXA	Dual energy x-ray absorptiometry
EGP	Endogenous glucose production
EMCL	Extra-myocellular lipid
FFM	Fat-free mass
FMI	Fat mass index
hs-CRP	High sensitivity C-reactive protein
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IMCL	Intra-myocellular lipid
M/I	Glucose infusion adjusting for circulating insulin concentrations
MRS	¹ H-magnetic resonance spectroscopy
Rd	Rate of disposal
RER	Respiratory exchange ratio
SAT	Subcutaneous adipose tissue
SCD1	Stearoyl-CoA desaturase 1
S _I	Insulin sensitivity
TA	Tibialis anterior
VAMP	Vesicle associated membrane protein
VAT	Visceral adipose tissue

Introduction

Type 2 diabetes and insulin resistance are more prevalent in populations of African origin than white populations [1, 2], but the main site of insulin resistance in obese black women is not known. Ectopic fat deposition in liver and skeletal muscle may differ by ethnicity [3, 4], resulting in organ-specific differences in insulin resistance. Whether this is related to tissue-specific alterations in insulin signalling among obese black women has, to our knowledge, not been studied.

Accordingly, in a sample of obese premenopausal black and white women, we sought to: (1) examine ethnic differences in hepatic and peripheral insulin sensitivity (S_I); (2) measure differences in hepatic and skeletal muscle lipid content and their association with S_I; and (3) measure the expression of genes involved in insulin signalling, fat oxidation and inflammation in skeletal muscle, and their ethnic-specific associations with S_I.

Methods

Participant selection This cross-sectional study included 30 obese premenopausal black and white women, matched for age (30–45 years) and BMI (≥ 30 kg/m²), with no known diseases, not pregnant or lactating, and who consumed <20 g alcohol/day. The study was undertaken in

accordance with the guidelines of The Declaration of Helsinki and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee. Participants gave written informed consent prior to participation.

Testing procedures A questionnaire was administered to measure family history of type 2 diabetes, smoking, alcohol and dietary intake (food frequency) [5]. Physical activity was measured using actigraphy (ActiGraph LLC, Pensacola, FL, USA). Fat mass, fat-free mass (FFM), abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) areas were measured by dual energy x-ray absorptiometry (DXA, Discovery-W, software 12.7.3.7; Hologic, Bedford, MA, USA).

Fasting blood samples were drawn for metabolites, insulin and adipocytokines before a standard 75 g OGTT. On another day, a two-step euglycaemic (± 5 mmol/l), hyperinsulinaemic clamp, with 6,6-[²H₂]glucose isotope label was performed, with a 3 h low-dose insulin infusion (10 mU m⁻² min⁻¹), followed by a 2 h higher dose insulin infusion (40 mU m⁻² min⁻¹), with samples drawn and respiratory exchange ratio (RER) measured (Quark RMR, Cosmed, Rome, Italy) in the last 30 min of each period. Serum metabolites, insulin and adipocytokines were measured using standard techniques (Electronic Supplementary Material [ESM] [Methods](#)) and 6,6-[²H₂]glucose was measured using Agilent 6890 gas chromatograph and analysed using ChemStation software (Agilent Technologies, Palo Alto, CA, USA).

Hepatic, and intra- (IMCL) and extra-myocellular lipid (EMCL), and total lipid content of the soleus and tibialis anterior (TA) muscles of the calf were measured by ¹H-magnetic resonance spectroscopy (MRS) and MRI, respectively, using a 3 Tesla scanner (GE Healthcare, Global Diagnostic Imaging, Pewaukee, WI, USA).

A biopsy was taken from the vastus lateralis muscle from which RNA was extracted and the expression of genes (ESM Table 1) was measured using the Applied Biosystems 7900HT Fast Real-time PCR system using standard cycling conditions (Applied Biosystems, Foster City, CA, USA) and expressed relative to $\beta 2$ microglobulin.

Statistics Differences in participant characteristics were compared using χ^2 analysis, one-way analysis of variance and/or covariance, adjusting for fat mass index (FMI), which takes into account differences in height and fat mass between groups. Bivariate associations were explored using Pearson's correlation coefficients, which informed multiple regression analyses that included an interaction term (ethnicity \times independent variable). Data were analysed using STATA version 11.1 (StataCorp, College Station, TX, USA).

Results

Participant characteristics The obese white and black women were of similar age, BMI, FFM and VAT, but black women were shorter and had a greater % body fat and FMI (Table 1). The women performed similar daily physical activity and consumed similar amounts of dietary fat. More white than black women consumed alcohol and had a family history of diabetes (26.7 vs 6.7%, $p=0.087$). Serum adiponectin was higher in white than black women, but high sensitivity C-reactive protein (hs-CRP) and other circulating inflammatory markers (data not shown) were not different.

Liver fat tended to be higher in white than black women. Calf TA and soleus IMCL content were similar, but total soleus fat content was higher in black than white women. Skeletal muscle expression of genes involved in insulin signalling, glucose transport and fat oxidation did not differ between black and white women (ESM Table 1), nor did they correlate with any measure of skeletal muscle fat content.

Fasting glucose, insulin and NEFA concentrations did not differ by ethnicity. More white than black women had impaired fasting glucose (IFG; 26.7 vs 6.7 $p=0.142$) and impaired glucose tolerance (IGT; 26.7 vs 0%, $p=0.031$). While basal endogenous glucose production (EGP) was not different, white women had higher EGP and less EGP suppression than black women during the low-dose clamp. Only one white woman had incomplete suppression of EGP during the high-dose clamp. S_I and RER during the low-dose and high-dose clamps were similar between white and black women.

Correlates of insulin sensitivity In black women, body fat measures correlated negatively with hepatic and peripheral S_I , whereas in white women, only VAT correlated with M/I -high (Table 2). In black women only, liver fat correlated negatively with suppression of EGP, soleus fat correlated negatively with glucose infusion adjusting for circulating insulin concentrations (M/I)-low, and skeletal muscle IRS1, vesicle associated membrane protein (VAMP) and stearoyl-CoA desaturase 1 (SCD1) expression correlated positively with rate of disposal (Rd)-low and M/I -high. In both black and white women, serum adiponectin correlated positively with peripheral S_I .

Discussion

The major findings of our study were that obese white women had reduced hepatic S_I compared with obese black women, whereas peripheral S_I did not differ. Significant associations between ectopic fat accumulation and S_I were observed in obese black, but not white women, suggesting that obese black women are more sensitive to the effects of ectopic lipid deposition than obese white women.

Until recently, studies demonstrating ethnic differences in S_I between black and white women [1, 2] have only measured whole-body S_I . DeLany et al [6] recently showed similar levels of hepatic S_I , but lower peripheral S_I in young (22–24 years) normal-weight black vs white women. In contrast, in older obese women, we found that peripheral S_I did not differ, but white women had lower hepatic S_I than black women. Studies in the USA have consistently reported higher liver fat of white compared with black women [7], which is supported in part by our study. However, liver fat was associated with reduced hepatic and whole-body S_I in black, but not white women. This indication of increased sensitivity to ectopic lipid deposition confirms data in African-Americans showing that for a given level of liver fat, black women were more insulin resistant than white women [7].

Although there were no ethnic differences in IMCL or EMCL content, IMCL was associated with lower S_I during the low-dose clamp in black, but not white women. Studies from the USA that have shown similar [4] or lower [8] IMCL levels in black than white women, but have demonstrated associations with S_I in white women only [4, 8]. Differences between our study and others may relate to differences in methods used to measure S_I , or to differences in the accumulation of lipid byproducts. Supporting the latter, we showed that in black women only, S_I was associated with skeletal muscle SCD1 expression. SCD1 converts saturated fatty acids to monounsaturated fatty acids and increases triacylglycerol esterification, thereby attenuating the accumulation of lipid metabolites such as diacylglycerol and ceramide, which interfere with insulin signalling [9]. Despite no ethnic differences in the skeletal muscle expression of insulin signalling genes, we showed that IRS1 and VAMP expression were associated with increased S_I in black, but not white women. IRS1 is integral to insulin signalling, while VAMP is involved in insulin-stimulated GLUT4 translocation, and is upregulated in hyperinsulinaemia [10].

We used the state-of-the-art measures of S_I and ectopic fat deposition, which have not been performed previously in an obese black African population. NEFAs were not measured during the clamp, precluding measurement of adipose tissue S_I ; however, fasting and OGTT NEFA concentrations were not different between ethnicities (ESM Fig. 1). While the white women had a greater family history of type 2 diabetes and a higher prevalence of IFG and IGT, adjusting for these differences, or analysis of only women with normal glucose tolerance did not alter the main findings of this study. The paradox of higher hepatic S_I but similar EGP in black compared with white SA women may be explained by lower hepatic insulin clearance in obese, insulin resistant black women [11]. Future studies that also include measures of C-peptide are required. Other limitations include self-reported alcohol intake and failure to control for the phase of the menstrual

Table 1 Participant characteristics

Variable	Obese white (<i>n</i> =15)	Obese black (<i>n</i> =15)	<i>p</i> value	<i>p</i> adjust FMI
Age (years)	36±4	36±5	0.978	
Body composition				
BMI (kg/m ²)	35.2±3.5	37.9±5.1	0.106	
FFM (kg)	52.4±7.4	49.7±6.7	0.297	
Fat mass (kg)	42.5±6.1	47.1±9.8	0.131	
Body fat (%)	44.8±3.6	48.4±3.4	0.008	
FMI (kg/m ²)	15.4±2.2	18.1±3.6	0.017	
Waist (cm)	97.6±7.5	101.8±10.3	0.207	
VAT (cm ²)	170±40	179±45	0.590	0.646
SAT (cm ²)	534±89	596±101	0.085	0.903
Circulating proteins				
Adiponectin (mg/l)	4.4 (3.2–5.9)	2.7 (2.0–3.8)	0.025	0.022
hs-CRP (ng/ml)	4.4±2.2	5.2±2.8	0.425	0.983
Ectopic fat				
Liver fat (%)	3.6 (1.2–9.5)	1.5 (1.1–2.1)	0.077	0.065
TA IMCL	148 (90–274)	119 (42–143)	0.070	0.206
Sol IMCL	711 (507–1080)	925 (506–1600)	0.485	0.792
TA fat content (%)	6.7 (5.5–9.1)	9.4 (7.1–14.2)	0.028	0.083
Sol fat content (%)	10.8 (9.7–14.7)	15.5 (13.4–18.9)	0.003	0.074
Basal clamp				
Fasting glucose (mmol/l)	5.1±0.2	5.1±0.4	0.591	0.903
Fasting insulin (pmol/l)	64.4±5.4	90.7±39.1	0.028	0.188
EGP (mg min ⁻¹ [kg FFM] ⁻¹)	2.6 (2.0–3.8)	2.7 (1.8–2.9)	0.335	0.497
Fasting RER	0.73±0.04	0.77±0.07	0.076	0.084
Fasting RMR (kJ [kg FFM] ⁻¹ day ⁻¹)	134 (128–145)	132 (126–152)	0.702	0.767
Low-dose clamp				
Glucose-low (mmol/l)	4.6±0.3	4.6±0.3	0.671	0.716
Insulin-low (pmol/l)	134.1±42.6	158.4±35.2	0.100	0.237
EGP-low (mg min ⁻¹ [kg FFM] ⁻¹)	1.9 (1.6–2.7)	0.8 (0–1.8)	0.006	0.002
Suppression of EGP (%)	17 (5–51)	56 (29–100)	0.006	0.002
<i>M</i> -low (mg min ⁻¹ [kg FFM] ⁻¹)	0.75 (0.39–1.98)	1.19 (0.33–3.73)	0.757	0.187
<i>M</i> / <i>I</i> -low (mg min ⁻¹ [kg FFM] ⁻¹ mmol/l ⁻¹)	0.31 (0.15–0.80)	0.39 (0.09–0.91)	0.871	0.320
Rd-low (mg min ⁻¹ [kg FFM] ⁻¹)	3.0 (2.4–4.1)	2.5 (2.0–3.7)	0.178	0.418
RER-low	0.75±0.06	0.77±0.06	0.317	0.218
RMR-low (kJ [kg FFM] ⁻¹ day ⁻¹)	136 (129–142)	137 (129–145)	0.791	0.712
High-dose clamp				
Glucose-high (mmol/l)	4.4±0.4	4.6±0.3	0.300	0.376
Insulin-high (pmol/l)	513.0±88.5	589.0±133.4	0.077	0.149
<i>M</i> -high (mg min ⁻¹ [kg FFM] ⁻¹)	9.3 (5.7–12.2)	8.5 (4.8–12.2)	0.962	0.631
<i>M</i> / <i>I</i> -high (mg min ⁻¹ [kg FFM] ⁻¹ mmol/l ⁻¹)	0.82 (0.70–1.36)	0.76 (0.58–0.93)	0.462	0.898
RER-high	0.83±0.06	0.85±0.08	0.573	0.457
RMR-high (kJ [kg FFM] ⁻¹ day ⁻¹)	139 (133–153)	137 (130–156)	0.964	0.731

For non-normally distributed data, values are median (IQR), with *p* values for log₁₀-transformed data. For normally distributed data, values are means±SD

EGP, calculated as the rate of appearance of glucose minus the glucose infusion rate; High-dose clamp, high-dose (40 mU m⁻² min⁻¹) insulin infusion; Low-dose clamp, low-dose (10 mU m⁻² min⁻¹) insulin infusion; *M*, glucose infusion rate, which reflects whole-body insulin sensitivity; RMR, resting metabolic rate; Sol, soleus; Suppression of EGP, calculated as the % change in EGP between baseline and the low-dose insulin infusion

Table 2 Correlates of hepatic and peripheral insulin sensitivity

Variable	% Suppression of EGP		Rd-low		M/I-low		M/I-high	
	White	Black	White	Black	White	Black	White	Black
Body composition								
Body fat (kg)	0.08	-0.64*	-0.09	-0.34	0.01	-0.64*	0.12	-0.60*
Waist	0.09	-0.65**	-0.18	-0.73**	-0.25	-0.67**	-0.44	-0.73*
VAT	-0.04	-0.52*	-0.20	-0.35	-0.34	-0.62*	-0.62*	-0.30
SAT	0.19	-0.54*	-0.21	-0.26	0.13	-0.45	0.17	-0.50*
Ectopic fat								
Liver fat	0.28	-0.57*	-0.44	-0.41	-0.45	-0.34	-0.53	-0.21
Soleus IMCL	0.59*	-0.46	-0.16	-0.52	0.12	-0.62*	-0.08	-0.46
Soleus fat (%)	0.34	-0.56*	-0.04	-0.40	0.27	-0.66*	0.09	-0.33
Muscle gene expression								
IRS	-0.32	0.49	0.04	0.65*	-0.15	0.56*	-0.37	0.66*
VAMP	-0.37	0.43	-0.07	0.68**	-0.18	0.52	-0.49	0.64*
SCD1	-0.33	0.49	-0.03	0.90**	-0.23	0.53	-0.29	0.72**
Circulating adipokines								
Adiponectin	0.41	0.49	0.47	0.77**	0.64**	0.56*	0.75**	0.71**

Values are Pearson correlation coefficients

* $p < 0.05$, ** $p < 0.01$

M/I-high, M/I corrected for circulating insulin levels during the high-dose ($40 \text{ mU m}^{-2} \text{ min}^{-1}$) clamp; M/I-low, M/I corrected for circulating insulin levels during the low-dose clamp; Rd-low, Rd during the low-dose ($10 \text{ mU m}^{-2} \text{ min}^{-1}$) clamp

cycle, which may have confounded our results. Further, we only included obese women; therefore these results cannot be extrapolated to non-obese women, or to men.

In conclusion, we found that although whole-body S_I was not different between obese black and white women, obese white women presented with lower hepatic S_I compared with obese black women. Notably, ectopic fat accumulation was associated with reduced S_I in black, but not white women. Future studies are required to gain an understanding of why black women are more sensitive to the effects of ectopic fat deposition than white women.

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