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Metabolism Clinical and Experimental 59 (2010) 1341 – 1350
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Ethnic differences in serum lipoproteins and their determinants in South African women

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Received 21 August 2009; accepted 15 December 2009

Abstract

The objective of the study was to characterize ethnic differences in lipid levels and low-density lipoprotein (LDL) particle size and subclasses in black and white South African women and to explore the associations with insulin sensitivity (S_I), body composition, and lifestyle factors. Fasting serum lipids and LDL size and subclasses, body composition (dual-energy x-ray absorptiometry), and S_I (frequently sampled intravenous glucose tolerance test) were measured in normal-weight (body mass index <25 kg/m^2) black (n = 15) and white (n = 15), and obese (body mass index >30 kg/m^2) black (n = 13) and white (n = 13) women. Normal-weight and obese black women had lower triglycerides (0.59 ± 0.09 and 0.77 ± 0.10 vs 0.89 ± 0.09 and 0.93 ± 0.10 mmol/L, $P < .05$) and high-density lipoprotein cholesterol (1.2 ± 0.1 and 1.1 ± 0.1 vs 1.7 ± 0.1 and 1.6 ± 0.3 mmol/L, $P < .01$) than white women. The LDL particle size was not different, but obese black women had more LDL subclass IV (17.3% ± 1.0% vs 12.5% ± 1.0%, $P < .01$). In white women, triglycerides and LDL particle size correlated with S_I ($P < .01$), whereas cholesterol levels correlated with body fat ($P < .05$). Low socioeconomic status, low dietary protein intake, and injectable contraceptive use were the major determinants of unfavorable lipid profiles in black women. Black women had lower triglyceride and high-density lipoprotein cholesterol levels and more small dense LDL particles than white women. The major determinants of serum lipids in black women were socioeconomic status and lifestyle factors, whereas in white women, S_I and body composition most closely correlated with serum lipids.
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1. Introduction

Obesity, visceral adipose tissue (VAT) accumulation, and insulin resistance are typically associated with dyslipidemia, characterized by reduced high-density lipoprotein (HDL) cholesterol levels, elevated triglyceride levels, and increased small dense low-density lipoprotein (LDL) particles [1,2]. These lipoprotein abnormalities are linked to an increased risk for atherosclerosis and cardiovascular disease [3].

Black women in South Africa and African Americans have been shown to have a less atherogenic lipid profile than white women, characterized by low triglyceride, total cholesterol, and LDL cholesterol levels [4-6]. As such, the age-standardized cholesterol-attributable mortality estimates were significantly lower in black than white South African women in 2000 (47 vs 152 deaths per 100 000) [7]. This

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doi:10.1016/j.metabol.2009.12.018

phenotype in black women may be attributed to their relatively low levels of VAT [5], as for the same level of body fatness, black women have less VAT than white women [4,8]. Paradoxically, black women are more insulin resistant than their white counterparts [9,10], suggesting that the association between insulin resistance and lipid metabolism may be different in persons of differing ethnic background. Indeed, Sumner et al [11] demonstrated that triglyceride levels, triglyceride to HDL ratio, and LDL particle size were not associated with insulin sensitivity in African Americans. Furthermore, for a given triglyceride level, African Americans were more insulin resistant than their white counterparts [6].

Although LDL cholesterol levels are low in black women [4], recent evidence suggests that the quality rather than only the quantity of LDL imparts increased cardiovascular risk [12]. The LDL comprises at least 4 distinct subclasses that differ in size, density, and metabolic characteristics, with small dense LDL being associated with increased cardiovascular risk [13]. Although insulin resistance is not typically associated with increased LDL cholesterol levels, greater concentrations of small dense LDL and lower levels of large LDL are concomitantly associated with reduced insulin sensitivity and increased adiposity [1,2]. Notably, LDL particle size or subclasses and their relationship with insulin resistance have not been compared in black and white Southern Africans.

Therefore, the aims of the study were 3-fold: (1) to characterize ethnic differences in lipid levels and LDL particle size and subclasses in black and white South African women of similar body fatness; (2) to explore the putative associations between insulin sensitivity, body composition, and lifestyle factors vs lipid levels; and (3) to examine the consistency of the relationships between lipid levels and LDL particle size between ethnic groups.

2. Methods

2.1. Subjects

The study population consisted of 15 normal-weight (body mass index [BMI] 18-25 kg/m^2) black, 15 normal-weight white, 13 obese (BMI >30 kg/m^2) black, and 13 obese white South African women. All participants were recruited by advertisement in local newspapers and from local church groups, community centers, and universities, as described previously [10]. Inclusion criteria were as follows: (1) age from 18 to 45 years; (2) no known diseases or taking medication for dyslipidemia, diabetes, hypertension, HIV/AIDS, or any other metabolic disorders; and (3) not currently pregnant, lactating, or postmenopausal. The study was approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. Before participating in the study, procedures and risks were explained to the subjects, all of whom gave written informed consent to participate in the study.

2.2. Testing procedures

2.2.1. Assessment of socioeconomic status, dietary intake, and physical activity

A demographic questionnaire was administered that included measures of socioeconomic status based on asset index, housing density, education, and employment. Asset index was based on 14 items reflecting individual and household wealth. Education was categorized by grades passed, and *housing density* was defined as the number of persons per room living in the household. Subjects were categorized as unemployed, students, informally employed, or employed. Based on a rank sum of these scores, a socioeconomic status score was devised that showed good internal reliability (standardized Cronbach $\alpha = 0.829$).

Dietary intake was estimated using a validated food frequency questionnaire. The questionnaire, administered by a registered dietician, comprises 100 food items with food photographs to determine portion size. Nutrient intake was calculated by means of the software program FoodFinder III, supplied by the Nutrition Intervention Research Unit (South African Medical Research Council, Parow, South Africa). Physical activity energy expenditure was characterized using the Global Physical Activity Questionnaire, and total physical activity energy expenditure was calculated in metabolic equivalents.

2.2.2. Body composition

Basic anthropometric measurements, including weight, height, and waist circumference (at the level of the umbilicus), were made. Body fat percentage was measured using dual-energy x-ray absorptiometry (Discovery-W, Software version 4.40; Hologic, Bedford, MA), the measurement of which has a coefficient of variation of 1.7%. Manually determined gynoid region of interest was determined for each individual by the same operator as previously described [14]. Visceral and subcutaneous adipose tissue (SAT) area was measured at the level of L4-L5 lumbar vertebrae using computed tomography (Toshiba X-press Helical Scanner, Tokyo, Japan).

2.2.3. Metabolic measures

After an overnight fast, a blood sample was taken for the determination of fasting glucose, insulin, lipids, and LDL particle size and subfractions. Subjects then underwent an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) to quantify insulin sensitivity. Baseline samples were drawn at −15, −5, and −1 minute before the infusion of glucose (50% dextrose; 11.4 g/m^2 body surface area) over 60 seconds at time 0. At 20 minutes, human insulin (0.02 U/kg; Actrapid, Novo Nordisk, Sandton, South Africa) was infused over 5 minutes at a constant rate. Plasma glucose and serum insulin concentrations were measured in the 3 baseline samples and the 32 samples drawn over 240 minutes after commencement of the glucose infusion. Glucose and insulin data from the FSIGT were used to calculate the insulin sensitivity index (S_I) using the minimal model of glucose kinetics of Bergman et al [15].

2.2.4. Blood pressure and carotid intima-media thickness

Blood pressure was measured in a seated position after 5 to 10 minutes of rest using an automated blood pressure monitor (Omron 711; Omron Health Care, Hamburg, Germany). The average of 3 measurements taken at 1-minute intervals was used in the analysis. Bilateral common carotid intima-media thickness (IMT) was measured as described previously [16]. In brief, IMT was measured at the point of the greatest thickness and at 1 cm upstream and 1 cm downstream of that point. *Mean IMT* was defined as the mean of 6 measurements, and the *maximum IMT* was defined as the largest of 6 measurements.

2.3. Biochemical analysis

Plasma glucose concentrations were determined using the glucose oxidase method (YSI 2300 STAT Plus; YSI, Yellow Springs, OH). Serum insulin concentrations were determined by immunochemiluminometric assays using the ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY). The intraassay and interassay coefficients of variation for plasma glucose and serum insulin concentrations were 0.6% and 2.5%, and 4.5% and 12.2%, respectively. Blood lipids were analyzed using the Roche Modular autoanalyzer (Penzberg, Germany). Enzymatic colorimetric assays were used to analyze total cholesterol, triglyceride, and HDL cholesterol concentrations. The LDL cholesterol concentrations were determined using the Friedewald formula.

Low-density lipoprotein size and subclass distribution were measured using nondenaturing polyacrylamide gradient gel electrophoresis of serum at 10°C to 14°C in 2% to 16% polyacrylamide gradient gels. Gels were subjected to electrophoresis for 24 hours at 125 V in Tris borate buffer (pH 8.3). Gels were fixed and stained for lipids in a solution containing oil red O in 60% ethanol at 55°C. Gels were placed on a light source and photographed using a luminescent image analyzer, LAS-3000 of Fujifilm (Kaisei Town, Japan), detection using white transmitted light source. Migration distance for each absorbance peak was determined; and the molecular diameter corresponding to each peak was calculated from a calibration curve generated from the migration distance of size standards of known diameter, which includes carboxylated latex beads (Duke Scientific, Palo Alto, CA), thyroglobulin, and apoferritin (HMW Std; Pharmacia, Piscataway, NJ) having molecular diameter of 380, 170, and 122 Å, respectively, and lipoprotein calibrators of previously determined particle size. The LDL subclass distribution (LDL I, IIA, IIB, IIIA, IIIB, IVA, and IVB) as percentage of total LDL was calculated as previously described [17].

2.4. Statistics

Results are presented as means ± standard error. The S_I, fasting insulin, triglyceride, and HDL cholesterol levels and IMT were normalized by log transformation and are presented as geometric means and fifth and 95th percentile

Table 1
Subject characteristics

	Normal-weight black (n = 15)	Normal-weight white (n = 15)	Obese black (n = 13)	Obese white (n = 13)
Age (y)	24 ± 2[c]	26 ± 2[d]	29 ± 2[c]	31 ± 2[d]
Body composition				
BMI (kg/m^2)	23.0 ± 0.9[C]	22.7 ± 0.9[D]	38.6 ± 1.0[C]	36.4 ± 1.0[D]
Body fat (%)	30.5 ± 1.3[C]	30.0 ± 1.3[D]	47.1 ± 1.4[C]	44.9 ± 1.4[D]
Waist circumference (cm)	76.3 ± 2.5[C]	80.1 ± 2.5[D]	114.0 ± 2.7[C]	109.1 ± 2.7[D]
VAT area (cm^2)	59 ± 10[C]	61 ± 10[D]	108 ± 12[B,C]	143 ± 11[B,D]
SAT area (cm^2)	170 ± 20[C]	183 ± 20[D]	607 ± 23[B,C]	497 ± 23[B,D]
Gluteal fat (kg)	1.7 ± 0.1[C]	1.7 ± 0.2[D]	4.1 ± 0.3[C]	3.5 ± 0.2[D]
Lifestyle factors				
Socioeconomic status score	12.1 ± 0.9[A]	22.4 ± 0.9[A]	12.1 ± 0.9[B]	23.1 ± 0.9[B]
Oral contraception (n [%])	0[A]	3 (20.0%)[A]	0[B]	2 (15.4%)[B]
Injectable contraception (n [%])	6 (40.0%)[A]	0[A]	3 (15.4%)[B]	1 (7.7%)[B]
Smokers (%)	3 (20.0%)	4 (26.7%)	2 (15.4%)	4 (30.8%)
PAEE (METS/d)	394 ± 92	281 ± 6	331 ± 96	411 ± 96
Energy intake (mJ)	11.9 ± 0.1	10.1 ± 0.1	13.4 ± 0.1	10.4 ± 0.1
Protein (%E)	12.4 ± 0.1	14.3 ± 0.1	11.5 ± 0.1[B]	16.7 ± 0.1[B]
Carbohydrate (%E)	52.9 ± 2.0	51.1 ± 2.1	49.8 ± 2.1	48.1 ± 2.1
Fat (%E)	32.7 ± 1.6	30.0 ± 1.8	37.8 ± 1.8[B]	31.0 ± 1.8[B]
Saturated fat (%E)	8.7 ± 0.6[c]	9.6 ± 0.6	10.6 ± 0.6[c]	11.0 ± 0.6
Monounsaturated fat (%E)	10.8 ± 0.7	10.8 ± 0.8	12.4 ± 0.8	10.3 ± 0.8
Polyunsaturated fat (%E)	10.6 ± 0.6[A]	6.2 ± 0.7[A]	11.8 ± 0.7[B]	6.6 ± 0.7[B]

Values are unadjusted mean ± SEM. All P values adjusted for age. PAEE indicates physical activity energy expenditure; METS, metabolic equivalents; %E, percentage of total energy intake.

[A] $P < .01$: normal-weight black vs normal-weight white.

[B] $P < .01$: obese black vs obese white.

[c] $P < .05$ and [C]$P < .01$: normal-weight vs obese black.

[d] $P < .05$ and [D]$P < .01$: normal-weight vs obese white.

Table 2
Metabolic parameters

	Normal-weight black (n = 15)	Normal-weight white (n = 15)	Obese black (n = 13)	Obese white (n = 13)
Insulin sensitivity				
Fasting glucose (mmol/L)	4.4 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.7 ± 0.1
Fasting insulin (mU/L)*	7.3 (2.1-19.6)^{C}	4.7 (3.2-12.8)^{D}	14.5 (5.5-28.2)^{B,C}	6.8 (2.4-12.7)^{b,d}
S_I ($\times 10^{-4}$ min^{-1}/[mU/L])*	1.9 (0.5-6.0) ^{A}	5.2 (1.5-8.3)^{A}	1.1 (0.6-6.1) ^{B}	3.2 (1.0-7.4)^{b}
Serum lipids				
Triglycerides (mmol/L)*	0.53 (0.30-1.10)^{a}	0.80 (0.4-1.8)^{a}	0.71 (0.40-1.30)	0.88 (0.5-1.5)
Total cholesterol (mmol/L)	3.6 ± 0.6	4.1 ± 0.2^{d}	3.8 ± 0.3^{B}	4.9 ± 0.3^{b,d}
HDL cholesterol (mmol/L)*	1.2 (0.7-2.0)^{A}	1.7(1.0-2.3) ^{A}	1.1 (0.7-1.7)^{b}	1.5 (0.7-2.3) ^{b}
LDL cholesterol (mmol/L)	2.1 ± 0.2	2.0 ± 0.2^{d}	2.3 ± 0.3	2.9 ± 0.3^{d}
Non-HDL cholesterol (mmol/L)	2.3 ± 0.2	2.4 ± 0.2^{d}	2.7 ± 0.3	3.4 ± 0.3^{d}
Total LDL particle size (Å)	276 ± 2	279 ± 2	274 ± 3	275 ± 2
Blood pressure				
Systolic (mm Hg)	104 ± 2	111 ± 3	112 ± 7	112 ± 4
Diastolic (mm Hg)	69 ± 1^{C}	70 ± 3	78 ± 3^{C}	75 ± 3
IMT				
Mean IMT (mm)*	0.36 (0.30-0.50)^{C}	0.38 (0.30-0.47)^{D}	0.44 (0.30-0.72)^{C}	0.48 (0.40-0.70)^{D}
Maximum IMT (mm)*	0.36 (0.30-0.50)^{C}	0.41 (0.30-0.50)^{D}	0.50 (0.3-1.00) ^{C}	0.52 (0.40-0.80)^{D}

Values are unadjusted mean ± SEM. All P values adjusted for age.
^{C} $P < .01$: normal-weight vs obese black.
^{a} $P < .05$ and $^{A}P < .01$: normal-weight black vs normal-weight white.
^{b} $P < .05$ and $^{B}P < .01$: obese black vs obese white.
^{d} $P < .05$ and $^{D}P < .01$: normal-weight vs obese white.
* Values are geometric mean (fifth and 95th percentile intervals), with P level on log-transformed data.

intervals. Two-way analysis of covariance, adjusting for age, was used to compare demographic, anthropometric, dietary, and metabolic measures between normal-weight and obese black and white women, with Fisher least significant difference post hoc analyses. Mann-Whitney U nonparametric statistics were used to compare physical activity and its components between ethnic and BMI groups. Pearson correlation coefficients were used to explore the relationships between measures of serum lipids, body composition, and lifestyle factors. Multiple linear regression was used to explore the independent associations between serum lipoprotein levels and more than 2 other variables. Data were analyzed using STATISTICA version 8 (Statsoft, Tulsa, OK).

3. Results

3.1. Subject characteristics according to BMI and ethnicity

The characteristics of the subjects are summarized in Table 1, with portions previously published [10]. Obese women were significantly older than normal-weight women ($P < .001$). Consequently, all subsequent analyses were adjusted for differences in age. As expected, obese women had higher levels of total, abdominal, and gluteal body fat than normal-weight women. Although there were no ethnic differences in BMI, percentage body fat, waist circumference, or gluteal fat, obese black women had significantly less VAT and more SAT than their white counterparts.

Black women were of a significantly lower socioeconomic status based on a composite score including asset index, education, employment, and housing density. The majority of the black (68%) and white (79%) women were not using any contraceptives. Of those using contraceptives, black women only used injectables, whereas the majority of white women used oral contraceptives. There were no significant differences between groups in the proportion of women who currently smoked. Total activity energy expenditure and dietary energy intake were also not significantly different between groups. However, reported dietary protein intake was lower and dietary fat, in particular

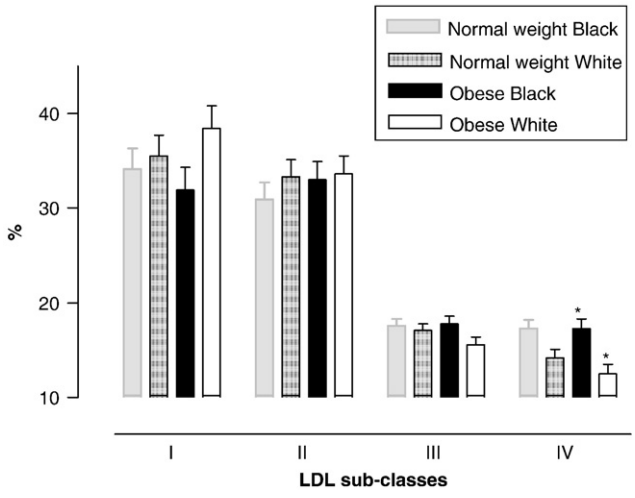

Fig. 1. Low-density lipoprotein subclasses in normal-weight and obese black and white women. *$P < .05$ for obese black vs white women.

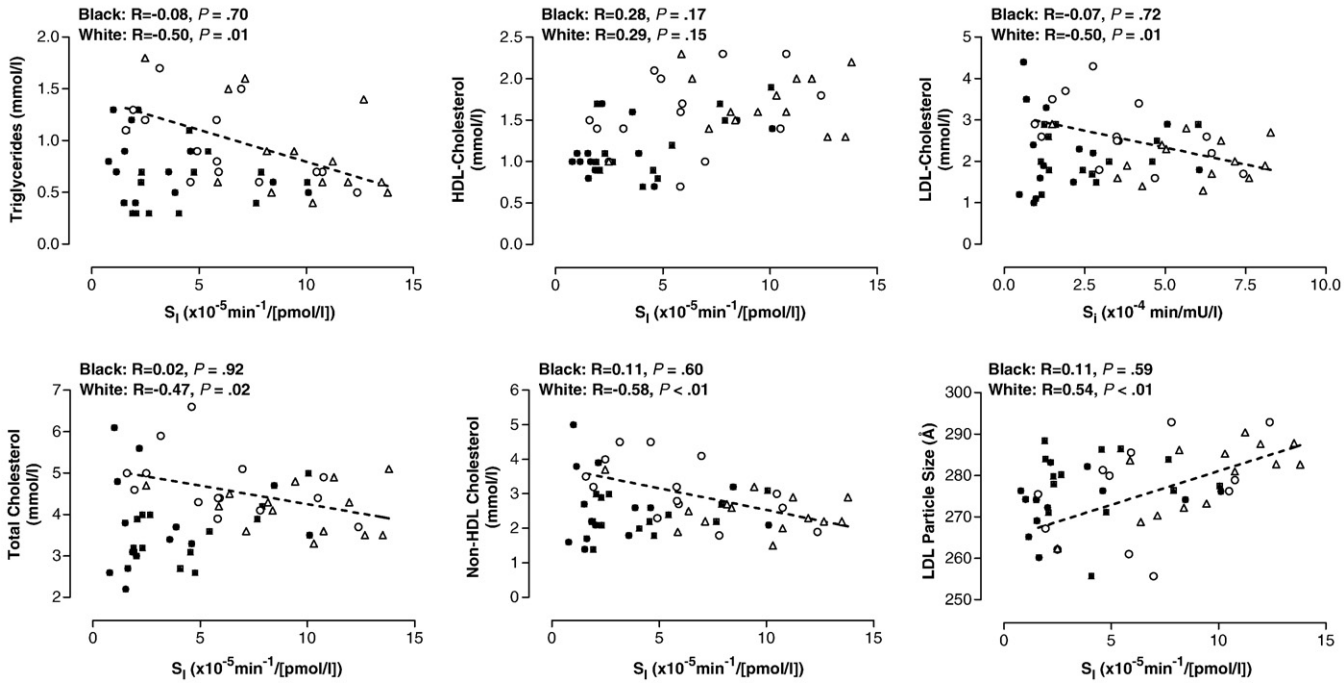

Fig. 2. Relationships between S_I and serum lipids in normal-weight (triangles) and obese (circles) black (closed symbols) and white (open symbols) women. Broken regression lines are for white women. No regression line is shown for relationships that are not significant.

polyunsaturated fat intake, was higher in obese black compared with obese white women.

3.2. Metabolic parameters according to BMI and ethnicity

Fasting glucose levels were not different between groups; but fasting insulin concentrations were significantly higher in obese women, with black women having significantly higher levels than white women (Table 2). Insulin sensitivity was significantly lower in black compared with white women, as reported previously [10]. Total, LDL, and non-HDL cholesterol levels were higher in obese white, but not black, women. Serum lipid levels were lower in black than white women, with significant ethnic differences observed for triglyceride and HDL cholesterol levels between normal-weight women and for total and HDL cholesterol levels between obese women (Table 2). There were no differences in LDL particle size between groups; but obese black women had significantly more small dense LDL (subclass IV) than obese white women (Fig. 1), independent of differences in S_I (P = .001). Similarly, no ethnic differences in blood pressure or IMT were observed; but obese women had higher diastolic blood pressure and IMT than normal-weight women.

3.3. Relationships between serum lipids, LDL particle size, and insulin sensitivity

Insulin sensitivity index correlated significantly with serum triglycerides, total cholesterol, non-HDL cholesterol, and LDL particle size in white women (Fig. 2); but only the associations with triglycerides (P = .016), non-HDL

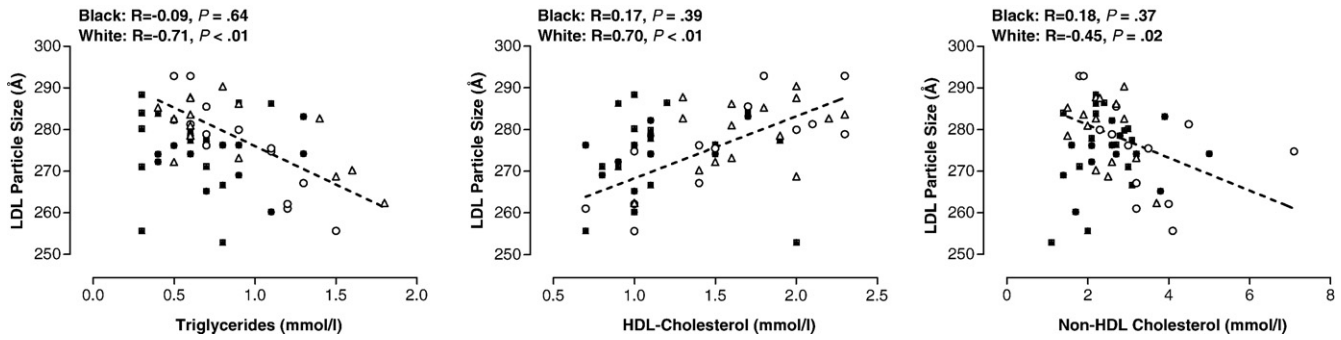

Fig. 3. Relationships between serum lipids and LDL particle size in normal-weight (triangles) and obese (circles) black (closed symbols) and white (open symbols) women. Broken regression lines are for white women. No regression line is shown for relationships that are not significant.

Table 3
Associations between serum lipids, body composition, and lifestyle factors in black and white South African women

	Ethnicity	Age (y)	Body fat (%)	Waist (cm)	SAT (cm^2)	VAT (cm^2)	Gluteal fat (%)	SES	Dietary fat (%E)	Dietary PUFA (%E)	Dietary protein (% E)	PAEE (MET/d)
Triglycerides (mmol/L)	Black	$r = 0.34$	$r = 0.33$	$r = 0.39$*	$r = 0.37$	$r = 0.36$	$r = -0.25$	$r = -0.09$	$r = 0.05$	$r = -0.24$	$r = -0.02$	$r = -0.07$
	White	$r = 0.20$	$r = 0.28$	$r = 0.24$	$r = 0.25$	$r = 0.29$	$r = -0.58^{\dagger}$	$r = 0.20$	$r = -0.20$	$r = -0.41$*	$r = -0.19$	$r = 0.14$
Total cholesterol (mmol/L)	Black	$r = 0.34$	$r = 0.25$	$r = 0.05$	$r = 0.11$	$r = 0.23$	$r = -0.35$	$r = 0.42$*	$r = -0.27$	$r = -0.39$*	$r = 0.28$	$r = -0.32$
	White	$r = 0.10$	$r = 0.37$	$r = 0.46$*	$r = 0.42$*	$r = 0.23$	$r = -0.15$	$r = 0.03$	$r = -0.10$	$r = -0.07$	$r = 0.09$	$r = -0.13$
HDL cholesterol (mmol/L)	Black	$r = -0.02$	$r = -0.04$	$r = -0.31$	$r = -0.26$	$r = -0.27$	$r = 0.05$	$r = 0.40$*	$r = -0.20$	$r = -0.35$	$r = 0.50^{\dagger}$	$r = -0.10$
	White	$r = -0.08$	$r = -0.19$	$r = -0.21$	$r = -0.13$	$r = -0.22$	$r = 0.39$	$r = -0.17$	$r = -0.03$	$r = 0.10$	$r = -0.01$	$r = -0.08$
LDL cholesterol (mmol/L)	Black	$r = 0.31$	$r = 0.26$	$r = 0.11$	$r = 0.16$	$r = 0.29$	$r = -0.36$	$r = 0.31$	$r = -0.23$	$r = -0.24$	$r = 0.09$	$r = -0.33$
	White	$r = 0.10$	$r = 0.39$*	$r = 0.48^{\dagger}$	$r = 0.43$v	$r = 0.30$	$r = -0.20$	$r = 0.04$	$r = -0.05$	$r = -0.03$	$r = 0.09$	$r = -0.15$
Non-HDL cholesterol (mmol/L)	Black	$r = 0.38$*	$r = 0.24$	$r = 0.20$	$r = 0.24$	$r = 0.36$	$r = -0.41$*	$r = 0.30$	$r = -0.21$	$r = -0.27$	$r = 0.09$	$r = -0.30$
	White	$r = 0.08$	$r = 0.39$*	$r = 0.48^{\dagger}$	$r = 0.45$*	$r = 0.32$	$r = -0.31$	$r = 0.08$	$r = -0.08$	$r = -0.10$	$r = 0.08$	$r = -0.10$
Total LDL particle size (Å)	Black	$r = -0.15$	$r = 0.13$	$r = -0.41$	$r = -0.09$	$r = 0.07$	$r = 0.20$	$r = 0.45$*	$r = 0.13$	$r = 0.14$	$r = 0.16$	$r = -0.37$
	White	$r = -0.14$	$r = -0.15$	$r = -0.15$	$r = -0.24$	$r = -0.21$	$r = 0.21$	$r = -0.24$	$r = -0.02$	$r = 0.33$	$r = -0.07$	$r = -0.24$
LDL subclass 1 (%)	Black	$r = 0.13$	$r = -0.13$	$r = -0.22$	$r = -0.18$	$r = 0.12$	$r = -0.08$	$r = 0.57^{\dagger}$	$r = -0.06$	$r = -0.27$	$r = 0.32$	$r = -0.24$
	White	$r = 0.01$	$r = 0.04$	$r = 0.20$	$r = 0.14$	$r = 0.08$	$r = 0.11$	$r = 0.01$	$r = -0.26$	$r = 0.19$	$r = -0.08$	$r = -0.10$
LDL subclass II (%)	Black	$r = 0.21$	$r = -0.04$	$r = 0.34$	$r = 0.26$	$r = 0.10$	$r = -0.23$	$r = -0.32$	$r = -0.11$	$r = -0.04$	$r = -0.18$	$r = 0.34$
	White	$r = 0.18$	$r = 0.08$	$r = -0.05$	$r = 0.05$	$r = 0.20$	$r = -0.16$	$r = 0.09$	$r = 0.26$	$r = -0.21$	$r = 0.07$	$r = 0.05$
LDL subclass III (%)	Black	$r = -0.28$	$r = -0.03$	$r = 0.07$	$r = 0.05$	$r = -0.27$	$r = 0.12$	$r = -0.41$*	$r = 0.13$	$r = 0.24$	$r = -0.17$	$r = 0.20$
	White	$r = 0.05$	$r = -0.10$	$r = -0.31$	$r = -0.29$	$r = -0.07$	$r = 0.29$	$r = -0.18$	$r = 0.33$	$r = 0.01$	$r = 0.31$	$r = 0.08$
LDL subclass IV (%)	Black	$r = -0.29$	$r = 0.09$	$r = -0.13$	$r = -0.03$	$r = -0.14$	$r = -0.17$	$r = -0.35$*	$r = 0.16$	$r = 0.38$*	$r = -0.25$	$r = -0.13$
	White	$r = -0.17$	$r = -0.32$	$r = -0.30$	$r = -0.46$	$r = -0.33$	$r = -0.17$	$r = -0.20$	$r = -0.20$	$r = -0.01$	$r = -0.25$	$r = 0.20$

Values are Pearson correlation coefficients. SES indicates socioeconomic status; PUFA, polyunsaturated fat intake.

* $P < .05$.

† $P < .01$.

cholesterol ($P = .012$), and LDL particle size ($P = .004$) were independent of body fatness. In contrast, S_I did not correlate with any of these lipid parameters in black women (Fig. 2). When exploring the relationship between serum lipid levels and LDL particle size, we found that LDL particle size correlated positively with HDL cholesterol levels and negatively with triglyceride and non-HDL cholesterol levels in white, but again not in black, women (Fig. 3).

3.4. Associations between serum lipids, body composition, and lifestyle factors

In white women, total, LDL, and non-HDL cholesterol levels correlated positively with total body fatness, waist circumference, and SAT, whereas in black women, triglyceride levels correlated with waist circumference (Table 3). Conversely, the proportion of gluteal fat (percentage of total body fat) was negatively associated with serum lipid levels, being significantly associated with serum triglyceride and non-HDL cholesterol levels in white and black women, respectively. Serum lipid levels did not correlate with age or any lifestyle factors in white women, apart from a negative correlation between serum triglyceride levels and polyunsaturated fat intake. In black women, the most significant correlate of lipid levels was socioeconomic status. Total cholesterol levels also correlated with polyunsaturated fat intake, whereas HDL cholesterol levels correlated with dietary protein intake, specifically that derived from animal sources ($r = 0.41$, $P = .03$) and not vegetable sources. The association between HDL cholesterol levels and socioeconomic status was no longer significant after adjusting for dietary protein intake ($\beta = 0.24$, $P = .20$ and $\beta = 0.40$, $P = .04$ for socioeconomic status and dietary protein intake, respectively). Black women using injectable contraceptives had significantly lower HDL cholesterol and triglyceride levels than women using no contraceptives ($P = .046$ and $P = .002$, respectively). However, the ethnic differences in serum triglyceride and HDL cholesterol levels reported in Table 2 persisted after adjusting for use of injectable contraceptives and dietary protein intake ($P = .026$ and $P = .013$, respectively).

4. Discussion

Despite relatively low triglyceride levels, black South African women had lower HDL cholesterol levels and a higher proportion of the smallest, most dense LDL subclass compared with white women. These ethnic differences could not be explained by lower VAT and/or lower insulin sensitivity in black women because serum lipid levels and LDL particle size did not correlate with these measures, as they did in white women. Rather, serum lipids and LDL particle size correlated with socioeconomic status, dietary protein intake, and contraceptive use in black women.

Studies undertaken in South Africa [4] and the United States [5,11,18] corroborate our finding of lower serum triglyceride levels in black compared with white women. However, the concomitantly lower HDL cholesterol levels in black women in this study, albeit in line with our previous work including a different cohort of more than 200 black South African women of similar tribal origin (Xhosa) residing in the same region [19], were still somewhat surprising, as other studies in South Africa have generally reported no ethnic differences in HDL cholesterol levels [4]. Interestingly, African Americans have been shown to have higher HDL cholesterol levels than their white counterparts [6]. It is important to note however that the HDL cholesterol levels of the black women in this and the previous study were still within the reference range.

For the first time in South Africa, ethnic differences in LDL particle size and density, important risk factors for cardiovascular disease [12,20], were explored. Despite differences in serum lipid levels and insulin sensitivity, LDL particle size was not different between ethnic groups. Low-density lipoprotein particle size is typically associated with elevated triglycerides and low HDL cholesterol levels [20], as demonstrated in the white women in this study. However, this was not the case in the black women, in whom no association between LDL particle size and serum lipids was found. This could perhaps be explained by the relatively low triglyceride and HDL cholesterol levels in black women, as these lipids provide substrates for the size reduction of LDL particles. The prevalence of small, dense LDL particles increases substantially as fasting plasma triglyceride levels exceed 1.69 mmol/L [21]. In both black and white women, serum triglyceride levels were not substantially elevated; and consequently, LDL particle size was within the reference range (<263 Å). Despite these findings, obese black women had a higher proportion of the smallest, most dense LDL subclass, which has been shown to have an independent predictive role for future cardiovascular and cerebrovascular events [12]. However, when correcting for the lower LDL cholesterol levels in black women, there were no differences in absolute amounts of small dense LDL between ethnic groups ($P = .483$). The cardiovascular risk implications of these findings, particularly in light of the relatively low HDL cholesterol levels in the context of low triglyceride levels in black women, are difficult to interpret and require longitudinal studies to resolve. Furthermore, despite ethnic differences in lipid levels, no differences were observed in blood pressure or IMT, the latter a marker of early atherosclerosis [22]. However, the IMT values were very low and overall in the reference range, as expected for these young, apparently healthy women. The value of IMT as a marker of vascular risk in young populations will only be known once more longitudinal studies are available.

High-density lipoprotein cholesterol levels and LDL particle size and subclasses in black women were positively associated with socioeconomic status based on a composite score of asset index, housing density, education, and employment. These findings correspond to those in other developing countries, in whom obesity and cardiovascular

risk factors are more prevalent in those of lower socioeconomic status living in urban areas (reviewed by Misra and Khurana [23]). The association between socioeconomic status and dyslipidemia is likely driven via its effects on lifestyle factors, in particular dietary intake. Indeed, we found that the association between socioeconomic status and HDL cholesterol levels was not independent of dietary protein intake, specifically animal-derived protein. Relatively low HDL cholesterol levels in a rural black South African population have previously been attributed to low dietary protein intake [24]. Low-protein, high-carbohydrate diets decrease HDL cholesterol levels by reducing the cholesterol content of HDL and hence increasing its clearance [25]. Within the South African context, socioeconomic status also influences contraceptive use, which we show here to be associated with HDL cholesterol and triglyceride levels in black women. A survey undertaken in the informal settlement where most of the black women in this study reside reported that 83% of women used injectable contraceptives, which was determined by the health care provider in more than 85% of cases [26]. Progestin-based injectable contraceptives (depot medroxyprogesterone acetate) have been associated with a reduction in HDL cholesterol and triglyceride levels [27]. Hence, this study identified factors related to socioeconomic status of the black women that may modify their lipid levels and likely impact on their risk for cardiovascular disease. Improvement in economic status alone will however not reduce the risk of cardiovascular disease, as results from the Transition and Health During Urbanization of South Africans study found that urbanization in black South African women was associated with an increase in total and LDL cholesterol levels, whereas increasing income was associated with increased intake of animal-derived protein, total and saturated fats, and dietary cholesterol, and decreased polyunsaturated to saturated fat ratios [28]. Furthermore, results from the INTERHEART Africa study showed that the risk for acute myocardial infarction was increased with higher income and education in the black African population [29]. Interventions aimed at reducing the risk of cardiovascular disease in South Africa should therefore also focus on increasing education regarding healthy nutrition and lifestyles.

Insulin resistance is typically associated with dyslipidemia, characterized primarily by elevated triglycerides, as well as reduced HDL cholesterol levels and increased small dense LDL particles, which are the direct/indirect result of decreased sensitivity to the inhibitory effects of insulin on very low-density lipoprotein secretion (reviewed by Avramoglu et al [30]). Accordingly, we found that serum lipids and LDL particle size correlated significantly with insulin sensitivity in white women (Fig. 2). However, this was not the case in black women, in whom there was a complete dissociation between insulin sensitivity and lipid levels. In accordance with our findings, Sumner et al [11] found that serum triglycerides, triglyceride to HDL cholesterol ratio, and LDL particle size did not decrease across tertiles for insulin sensitivity, measured using a FSIGT, in 125 African American women. Similar findings for serum triglycerides were reported in our cohort of 222 black South African women [31].

Ethnic differences in lipid levels and their association with insulin sensitivity cannot only be attributed to lifestyle factors, but could also relate to genetic differences in lipid metabolism. Indeed, a functional polymorphism within the lipoprotein lipase (LPL) gene promoter (−93t→g) has been shown to be present at a significantly higher frequency in a black South African population compared with a white Dutch population (76.4% vs 1.7%), with black subjects with the −93g allele having significantly lower triglycerides levels than those with the −93t allele [32]. Furthermore, African Americans have been shown to have higher LPL activity than their white counterparts [5,18], suggesting greater clearance of triglycerides from the circulation. Sumner et al [18] found that insulin resistance did not alter LPL activity in African American women, as demonstrated in other groups. We might therefore speculate that the relatively low levels of triglycerides in black women in this study might be explained by relatively high levels of LPL activity that are not altered by insulin resistance, but may in fact be up-regulated by the associated hyperinsulinemia. Future studies are required to explore this further.

An alternate explanation for the relatively low lipid levels in black compared with white women might be their lower propensity to accumulate VAT, as shown here and in other studies [4,5]. Indeed, Nieves et al [2] demonstrated that increased VAT was a more significant determinant of an atherogenic lipid profile than insulin resistance in apparently healthy individuals. Although obese black women in our study had less VAT than their white counterparts, serum lipid levels did not correlate with VAT in either black or white women. Rather, serum lipid levels were associated with total body fat, but more specifically abdominal SAT, in white women, whereas in black women, only a weak association between serum triglycerides and waist circumference was found. The primary source of free fatty acid delivery to the liver is from subcutaneous depots rather than from VAT, with greater postprandial free fatty acid delivery in upper-body vs lower body obesity [33], possibly explaining these findings. Conversely, it has been proposed that peripheral (gluteal-femoral) fat acts as a "sink" and is cardioprotective [34], possibly benefiting black women more than white women because of their greater peripheral fat distribution [35]. Indeed, we found that gluteal fat accumulation was negatively associated with serum lipid levels, in particular serum triglycerides and non-HDL cholesterol levels, in both black and white women, respectively, supporting previous findings in postmenopausal women [34]. Although black women tended to have a larger proportion of gluteal fat ($P = .058$) than white women, the association with serum lipid levels was not stronger than that in white women. These associations may however be confounded by the small sample size in this study and should be investigated in a larger cohort.

In conclusion, black South African women had lower triglyceride and HDL cholesterol levels and a higher proportion of the smallest, most dense LDL subclass than white women, despite being less insulin sensitive. Whereas lipid levels and LDL particle size correlated with insulin sensitivity and body fatness in white women, this was not the case in black women, in whom lipid levels were more closely associated with modifiable lifestyle factors. Notably, the prevalence of ischemic heart disease and stroke is higher in African Americans than white Americans, who present with a similar phenotype to black South African women [11,18]. The explanation for this conundrum may be negative effects of insulin resistance on, for example, inflammation, coagulation, and vascular reactivity. Whether this increases the risk of cardiovascular disease in black South African women, despite the relatively low triglyceride levels, remains to be analyzed in longitudinal studies.

Acknowledgment

We thank the research volunteers for their participation in this study; Nandipha Sinyanya for her excellent field work; Hendriena Victor, Judy Belonje, and Sacha West for their expert technical assistance; and Madelaine Carstens for performing the dietary analyses. Cornelia Zwimpfer is acknowledged for performing the LDL particle size measurements. Dr Jack Bergman and Naomi Fenton of Symington Radiology are thanked for performing the computed tomographic scans, and Linda Bewerunge is thanked for performing the dual-energy x-ray absorptiometry scans.

This study was funded by the Medical Research Council of South Africa, the International Atomic Energy Agency, the National Research Foundation of South Africa and Royal Society SA-UK Science Networks Programme, the University of Cape Town, and the United States Department of Veterans Affairs. There are no conflicts of interest.

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