

Ethnic differences in serum lipids and lipoproteins in overweight/obese African-American and white American women with pre-diabetes: significance of NMR-derived lipoprotein particle concentrations and sizes

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ABSTRACT

Objective: African-American women (AAW) suffer disproportionately from higher rates of cardiovascular disease (CVD) mortality compared with white American women (WAW), despite favorable lipid and lipoprotein profile. Therefore, we used nuclear magnetic resonance (NMR) to examine lipoprotein particle concentrations and sizes in overweight/obese AAW and WAW with pre-diabetes.

Participants and methods: We studied 69 AAW and 41 WAW, with mean age 46.5±11.3 years and body mass index (BMI) 37.8±6.4 kg/m². All participants completed standard oral glucose tolerance test (OGTT) and frequently sampled intravenous glucose tolerance test (FSIVGTT). Insulin sensitivity (Si) was calculated using MINIMOD method. Body composition was assessed using dual-energy X-ray absorptiometry (DEXA). Fasting blood was obtained for traditional lipids/lipoproteins and NMR-derived lipoprotein particle sizes and concentrations.

Results: We found that AAW with pre-diabetes were more obese (BMI 38.8±6.7 vs 36.0±5.4 kg/m², p=0.02) than WAW. Mean Si was not significantly different. However, the mean serum triglycerides were lower, whereas the high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A1 (Apo A1) were significantly higher in AAW versus WAW. The large HDL particle concentration (6.1±3.1 vs 4.6±3.1 μmol/L, p=0.02) was significantly higher in AAW versus WAW. Mean total very low-density lipoprotein (VLDL) particle concentration was lower in AAW versus WAW (39.9±24.4 vs 59.2±25.6 nmol/L, p<0.001). While mean total LDL particle concentrations were not different, mean small LDL particle concentrations were lower in AAW versus WAW (538.8±294.1 vs 638.4±266 nmol/L, p=0.07).

Conclusions: We found a more favorable NMR-derived lipoprotein profile in AAW that extends the traditional antiatherogenic lipid/lipoprotein profiles. Clinically, these favorable lipid/lipoprotein profiles cannot explain the paradoxically higher CVD mortality in AAW than WAW and warrant further prospective outcome studies.

Key messages

- Traditional lipid and lipoproteins are unlikely to explain the higher cardiovascular mortality in African American and White American Women with prediabetes.
- NMR derived lipoprotein particles and sizes provide a more extensive measure of cardiovascular risk. However, the favorable NMR derived lipoprotein profile cannot explain the excessive cardiovascular disease mortality in African American than White American women.
- Nontraditional cardiovascular disease risk factors maybe required to explain the excess cardiovascular disease mortality in African American than White American women.

INTRODUCTION

Cardiovascular diseases (CVDs) continue to emerge as the leading cause of deaths in the Western world. The recent observation of disparities in CVD mortality among ethnic/racial populations has raised concerns. Comparatively, African-Americans (AAs) are disproportionately affected by obesity and type 2 diabetes and the associated CVD morbidity and mortality compared with white Americans (WAs),¹⁻⁶ despite the more favorable lipid profile (higher high-density lipoprotein cholesterol (HDL-C)/lower triglyceride, more buoyant low-density lipoprotein (LDL) particle size)³⁻⁶ and greater insulin resistance in AA than in WA.⁷⁻¹⁰ Most of these studies measured lipids and lipoprotein levels using traditional enzymatic methods.^{3-5 9-11} Recently, detailed measurements of lipoprotein particle sizes and concentrations in AA and WA have been performed using nuclear magnetic resonance (NMR).¹²⁻¹⁷ The most striking observation found that HDL-C is inversely associated with

incident coronary heart disease in non-black populations, but not in blacks.^{16 17} However, NMR-derived HDL particles were inversely associated with incident coronary heart disease across all ethnic/racial populations.^{12 13 15–18} Hence, whether differences in NMR-derived lipoprotein particle concentrations and sizes could partly account for the ethnic differences in CVD mortality in AA versus WA remains uncertain. In this regard, plasma lipoproteins comprise varying concentrations of other protein coats surrounding the core of cholesterol and triglycerides.^{3 12 13 15–18} These differences result in several multiple particle sizes and subclasses of LDL, HDL and very low-density lipoprotein (VLDL), which have different biological and atherogenic properties.¹⁹ Previous studies have shown that while LDL-C enhances, HDL-C reduces atherosclerosis in several populations, except in black people of African ancestry living in diverse geographic locations.^{4 8–12 14–18} The exact reasons for this ethnic/racial disparity remain unknown, but it is believed to be partly genetic.^{20 21}

The advent of NMR technique has provided opportunity to examine in detail subclasses of the major lipoprotein particle concentrations and sizes.^{13–17} In this context, the atherogenic properties of the lipoproteins are determined by the overall biological activity of the individual subclasses. These lipoprotein particles are modified by several factors, including genetics, obesity, physical fitness, glucose dysregulation, insulin resistance, etc.^{10 12–15 19–21} Furthermore, several recent studies have shown racial/ethnic differences in the lipoprotein properties.^{12 14–18} Surprisingly, AAs have favorable lipids and lipoproteins but suffer from excess CVD mortality and morbidity than WAs, especially women.^{1–3 8–11} To further explain this paradox, we sought to perform comprehensive studies to examine (1) ethnic/racial differences in standard traditional lipids and lipoproteins and (2) NMR-derived lipoprotein particle concentrations and sizes in sedentary, overweight/obese AA and WA women with pre-diabetes.

PARTICIPANTS, MATERIALS AND METHODS

The study participants were recruited from local newspaper advertisement and our research population database. Each participant signed a written consent form approved by the Institutional Review Board of the Ohio State University Wexner Medical Center. A total of 248 participants were screened for the study. After screening, we recruited 110 overweight/obese women with pre-diabetes (69 AA and 41 WA; mean age 46.5±11.3 years; body mass index (BMI) 37.8±6.4 kg/m²). Pre-diabetes was defined as (1) impaired fasting glucose (IFG); 100–125 mg/dL, (2) impaired glucose tolerance (IGT); 2-hour glucose 140–199 mg/dL and (3) hemoglobin A1C (A1C); 5.7–6.4%. The following were excluded: (1) patients with severe liver, heart, lung and kidney diseases, (2) those participating in endurance exercise or regular competitive sports, (3) those participating in

weight reduction program within the past 6 months and (4) smokers.

CLINICAL AND ANTHROPOMETRIC MEASUREMENTS

Participants reported to Center for Clinical and Translational Science (CCTS) Clinical Research Center (CRC) after 10–12-hour overnight fast. Blood pressure (BP) was measured three times, at 10 min intervals, with the participant in supine position. The average of the three BPs was taken as the mean basal BP. Height (cm) and weight (kg) were used to calculate BMI (kg/m²); waist and hip circumference (cm) was used to calculate waist:hip ratio (WHR) in each participant. A whole-body dual-energy X-ray absorptiometry (DEXA) scan was conducted using the Lunar DPX Pro 2001 (Lunar Corp, Madison, Wisconsin, USA) with the participant lying supine on a flat surface. All the participants completed a physical activity and online Food Frequency Questionnaire by Viocare Inc.

METABOLIC STUDIES

Baseline fasting blood was obtained for serum insulin, c peptide, glucose and lipids/lipoproteins (total cholesterol, triglyceride, HDL-C, LDL-C, apolipoprotein A1 (Apo A1) and Apo B100). Serum blood samples were obtained for lipoprotein (HDL, LDL, VLDL) particle concentrations and sizes in each participant using NMR techniques. Patients underwent oral glucose tolerance test (OGTT) and frequently sampled intravenous glucose tolerance test (FSIVGTT) on two separate days at the CRC as previously described.⁷

Oral glucose tolerance test

Each participant was instructed to ingest at least 250 g of carbohydrate in their regular meals for at least 3 days before the test as previously described.^{7–9} Blood samples were drawn for serum glucose, insulin and c peptide levels. The participants then ingested 75 g of oral glucose load (Glucola, Baltimore, Maryland, USA) over a 2 min period. Blood samples were drawn at t=0, 30, 60, 90 and 120 min for serum glucose, insulin and c peptide levels.

Frequently sampled intravenous glucose tolerance test (FSIVGTT)

The participants returned to the General CRC on a separate day for FSIVGTT. With the participant in a supine position, two intravenous needles were inserted into the forearm veins and kept patent with 0.9% normal saline infusion as previously described.^{7–9} Briefly, four blood samples were obtained at t=−20, −10, −5 and 0 min for basal serum glucose, c peptide and insulin concentrations. The average of the four samples was considered the basal level. Thereafter, 0.3 g/kg glucose (50 mL of 50% dextrose water) was infused over a 1 min period. At t=19 min, intravenous insulin (0.05 units/kg, Humulin; Eli Lilly, Indianapolis, Indiana, USA) dissolved in 30 mL

of 0.9% normal saline was infused over 60 s. Blood samples were obtained at frequent intervals (t=2, 3, 4, 5, 6, 8, 10, 12, 16, 19, 22, 24, 25, 27, 30, 40, 60, 70, 90, 120, 140, 150, 160 and 180 min) for serum glucose, c peptide and insulin levels.

CALCULATIONS

BMI was used to define body weight as follows: overweight=25–29.9 kg/m² and BMI>30–35 kg/m². Nutritional analysis was performed using electronic Food Frequency Questionnaire by Viocare (<http://www.viocare.com/index.aspx>). Insulin resistance and β cell function were also calculated using the homeostasis model assessment (HOMA).^{7–9} HOMA insulin resistance index (HOMA-IR) was calculated as follows: fasting insulin (μ U/mL) \times fasting plasma glucose (mmol/mL)/22.5. In addition, Bergman's MINMOD Millennium 6.1 software program was used to calculate insulin sensitivity (Si) and various aspects of insulin dynamics.⁷

ANALYTICAL METHODS

All the blood samples were centrifuged at -4°C , the supernatant collected and stored at -20°C and -80°C . All the metabolic assays of each participant were run in a single batch to minimize interassay variability. The serum glucose levels were measured by glucose oxidase method (Model 2300, Yellow Springs Instrument, Antioch, Ohio, USA). Serum insulin and c peptide levels were measured by standard radioimmunoassay techniques. The coefficients of variation (CVs) were 6% and 10%, respectively. The lower limit of the c peptide assay was 0.1 ng/mL, and the intra-assay and interassay CVs were 7% and 13%, respectively. The A1C was measured by the cationic, microcolumn chromatography technique (Bayer). The normal reference range was 4.0–5.6% (20.2–37.7 mmol/mol). The serum cholesterol, HDL-C and triglycerides were measured using enzymatic methods. LDL-C was calculated using Friedwald's equation: $\text{LDL-C} = \text{total cholesterol} - \text{HDL-C} - \text{triglyceride}/5$, for serum triglycerides <400 mg/dL. The Apo A1 and Apo B100 were measured using ELISA (R&D Quantikine, Missouri, USA). Lipoprotein particle concentrations and sizes were measured using NMR (LipoScience, Raleigh, North Carolina, USA).

MEASUREMENTS OF LIPOPROTEIN PARTICLES AND SIZES USING NMR

Measurements of lipoprotein particles

The HDL, VLDL and LDL lipoprotein particle sizes (small, medium and large) and concentrations were measured using NMR as previously described.^{15–17}

Subclasses of lipoprotein particle sizes (nm)

These were derived from the NMR data as previously described.^{15–17}

- HDL (nm): small (7.3–8.2), medium (8.2–8.8) and large (8.8–13.0).
- VLDL (nm): small (27–35), medium (35–60) and large (>60).
- LDL (nm): small (18.0–21.2), medium (19.8–21.2) and large (21.2–23.0).

Lipoprotein particle concentrations

- Particle concentrations were obtained directly from the NMR-measured amplitudes of the distinct lipid methyl-derived signals as previously described.^{15–17}
- Weighted-average lipoprotein particle sizes are derived by the sum of the diameters of each subclass of NMR signals multiplied by its relative mass percentage based on the amplitude of methyl-derived NMR signal as previously described.^{15–17} The interassay CVs of lipoprotein particles were provided by the reference laboratory of LipoScience^{15–17}

STATISTICAL ANALYSES

Results are expressed as mean \pm SD, unless stated otherwise. Statistical analyses were performed using SAS V.9.1. The non-parametric data are analyzed using χ^2 and Mann-Whitney rank test. Student's unpaired t-test and multiple t-tests were used to analyze the data within and between the groups. Spearman's univariate linear regression was used to determine the relationships among Si, serum glucose, insulin, BMI and traditional lipids and lipoproteins and NMR-derived lipoprotein particles. Multiple regression analyses were performed using linear square regression models to examine the relationships between Si and cardiometabolic markers after adjusting for age, BMI and WHR. Probability $p < 0.05$ was considered to be statistically significant.

RESULTS

Clinical characteristics of obese AA and WA women with pre-diabetes

As shown in [table 1](#), the mean age was not significantly different in our overweight/obese AA and WA women with pre-diabetes (45.4 \pm 11 vs 46.4 \pm 12 years, $p=0.993$). We found that AA women with pre-diabetes were more obese (BMI 38.8 \pm 6.7 vs 36.0 \pm 5.4 kg/m², $p=0.02$), with higher per cent body fat (47 \pm 3% vs 46 \pm 2%, $p=0.04$). AA women had lower lean body mass compared with WA women. We found no statistically significant differences between the systolic BP, while the diastolic BP was statistically higher in AA than WA women (80.4 \pm 10.3 vs 77.1 \pm 7.5 mm Hg, $p=0.05$).

We estimated the daily caloric intake as well as the respective per cent calories derived from carbohydrate, fat and protein. We found that the estimated total daily calories tended to be higher but not significantly different in AA versus WA women with pre-diabetes (2635 \pm 1560 vs 2241 \pm 642 kcal/day, $p=0.009$). The per cent calories derived from carbohydrate were lower (43.61 \pm 18.30% vs 46.46 \pm 7.33%, $p=0.09$) in AA compared with

Table 1 Clinical and metabolic characteristics in overweight/obese African-American and white American women with pre-diabetes

Parameter	Group	African-Americans	White Americans	p Value
N	110	69	41	
Age (years)	46.5±11.3	45.4±11	46.4±12	0.993
Height (cm)	166.9±7.3	165±6	165±6	0.998
Weight (kg)	105.4±19.7	107.7±19.8	101.4±19.0	0.102
BMI (kg/m ²)	37.8±6.4	38.8±6.7	36±5.4	0.02
WHR (%)	0.92±0.7	0.9±0.1	1.0±0.1	0.009
BF (%)	45.9±4.9	47±3	46±2	0.044
LBM (%)	54.1±4.9	52±3	55±2	0.0477
SBP (mm Hg)	128.3±14.5	129.6±16.1	126.1±10	0.167
DBP (mm Hg)	79.2±9.6	80.4±10.39	77.1±7.5	0.05
A1C (%)	5.9±0.43	5.97±.40	5.67±0.40	0.0003
Si ($\times 10^{-4} \times \text{min}^{-1} [\mu\text{U/mL}]^{-1}$)	2.8±1.9	2.7±1.6	2.9±2.3	0.694
HOMA-IR	3.7±2.4	3.11±1.70	3.47±1.9	0.0009

Values are mean±SD.

A1C, hemoglobin A1C; BF, body fat; BMI, body mass index; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; LBM, lean body mass; SBP, systolic blood pressure; Si, insulin sensitivity; WHR, waist:hip ratio.

WA women. The per cent calories derived from protein were significantly lower in AA compared with whites (14.84±3.29% vs 16.81±2.993%, $p=0.001$), while per cent calories derived from fat were significantly higher in AA versus WA women with pre-diabetes (39.86±7.66% vs 35.38±7.07%, $p=0.001$). Parenthetically, the per cent calories derived from the macronutrients were similar to that of typical or standard American diet. Also, the estimated daily intake of saturated, monosaturated and polyunsaturated fatty acid was higher in AA compared with WA women, but the daily intake of total cholesterol was not different (data not shown).

As shown in table 2, we found that mean fasting serum glucose, insulin and c peptide were significantly lower in overweight/obese AA compared with WA women with pre-diabetes. Mean serum glucose levels were significantly lower at $t=30$ and 60 min in AA than WA. However, mean serum insulin levels were not different in AA versus WA after glucose challenge. In

contrast, the mean serum c peptide levels during OGTT were significantly lower in AA versus WA women with pre-diabetes (table 2). The mean Si was similar in our overweight/obese AA versus WA women (table 1). In contrast, HOMA-IR was significantly lower in AA than WA women with pre-diabetes (table 1). The mean incremental integrated glucose response was significantly lower in AA versus WA women with pre-diabetes (146.36±100.44 vs 200±111.13 mg/dL.min, $p=0.02$). In contrast, incremental integrated insulin (399.05±176.84 vs 420.89±196.68 $\mu\text{U/mL.min}$, $p=0.45$) and c peptide (31.60±9.48 vs 34.51±12.34 ng/mL.min, $p=0.25$) were not significantly different in AA versus WA women with pre-diabetes.

We examined the serum lipids and lipoproteins in overweight/obese AA and WA women with pre-diabetes measured using traditional methods (table 3). We found no significant differences in total serum cholesterol, LDL-C, HDL-C and non-HDL-C in our population.

Table 2 Serum glucose, insulin and c peptide responses during oral glucose tolerance test in overweight/obese African-American and white American women with pre-diabetes

OGTT	0 min	30 min	60 min	90 min	120 min
	Glucose		(mg/dL)		
African-Americans	96.6±12.1*	140.1±27.6**	141.3±36.3 [§]	128.4±38.7 ^α	123±39
White Americans	101.3±10.0	159.27±23.5	161.7±40	148.9±44.4	127.2±43.9
	Insulin		($\mu\text{U/mL}$)		
African-Americans	12.9±6.6 [^]	87.6±55.0	102.8±56.0	89.3±47.7	89±59
White Americans	15.6±7.8	82.0±43.1	107.8±68.4	98.7±47.1	103.7±81.7
	c Peptide		(ng/mL)		
African-Americans	2.69±0.9 [^]	8.9±3.6	11.3±3.6 [£]	11.1±3.1 ^α	10.9±3.8*
White Americans	3.4±1.4	10.0±3.8	13.1±4.1	13.8±3.9	10.8±5.0

Values are mean±SD.

AA versus WA: * $p=0.06$; [^] 0.0002 ; ^Ω <0.0001 ; ** 0.007 ; [§] 0.02 ; ^α 0.03 ; [^] 0.008 ; [£] 0.04 ; ^α 0.001 .

OGTT, oral glucose tolerance test.

Table 3 Serum fasting lipids and lipoproteins in overweight/obese African-American and white American women with pre-diabetes using traditional enzymatic methods

Parameter	Group	African-Americans	White Americans	p Value
N	110	69	41	
Cholesterol (mg/dL)	186.7±37.3	188±35	187±37	0.933
Triglycerides (mg/dL)	99.1±53.7	84.2±47.9	125±55.5	<0.0001
HDL-C (mg/dL)	50.8±13	52.6±12.9	48.2±13.2	0.08
LDL-C (mg/dL)	117±32	118.5±33.9	118.3±29.5	0.972
Cholesterol/HDL ratio	3.9±1.0	3.7±0.92	4.2±1.1	0.01
Non-HDL cholesterol (mg/dL)	137.9±34.9	135.8±36.7	143.2±32.2	0.287
Apo A1 (mg/dL)	149±25.9	153.6±24.5	143.2±32.2	0.03
Apo B100 (mg/dL)	92.3±22.4	91.1±24.5	94.9±19.9	0.405

Values are mean±SD.

Apo A1, apolipoprotein A1; Apo B100, apolipoprotein B100; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

However, the mean serum triglycerides were significantly lower in AA versus WA women (84.2±47.9 vs 125±55.6 mg/dL, $p<0.0001$). In addition, mean HDL-C tended to be higher in AA women compared with WA women (52.6±12.0 vs 48.2±13.2, $p=0.08$). We found statistically significant differences in Apo A1 (153.6±24.6 vs 142.7±27.1, $p=0.03$) but not Apo B100 (91±24 vs 94±19.1, $p=0.40$) levels in our AA and WA women with pre-diabetes.

Table 4 shows the NMR-derived mean particle sizes and concentrations in our overweight/obese AA and WA women with pre-diabetes. Mean total VLDL (44.9±6.04 vs 48.6±6.9 nm, $p=0.006$) was lower, while LDL (21.1±0.47 vs 20.7±0.54 nm, $p=0.03$) and HDL (9.2±0.5 vs 8.9±0.5 nm, $p=0.01$) particle sizes were significantly higher in AA versus WA women. The mean total LDL particle concentrations were not different in AA versus WA women (1226.2±383.8 vs 1248.2±286.5 nmol/L, $p=0.732$).

However, the mean small LDL particle concentration tended to be lower (537.8±291.1 vs 638.4±266.0 nmol/L, $p=0.06$), while the large LDL was not present in our AA versus WA women. The total HDL particle concentration was not different in AA versus WA women (33.9±5.8 vs 33.1±5.4 µmol/L, $p=0.473$). The mean concentration of the large HDL particles (6.1±3.2 vs 4.6±3.1 µmol/L, $p=0.02$) was significantly higher in AA versus WA women. However, the mean medium (10.7±5.4 vs 10.5±5.4 µmol/L, $p=0.864$) and small (17.1±4.9 vs 17.9±5.3 µmol/L, $p=0.407$) particles were not different in AA versus WA women with pre-diabetes.

VLDL is the major carrier of triglycerides in the circulation. Therefore, we measured the concentrations of different subclasses of VLDL in AA and WA women with pre-diabetes. As shown in table 4, the total VLDL particle concentration was significantly lower in AA compared with WA women (39.9±24.4 vs 59.2±25.6 nmol/L, $p<0.001$).

Table 4 NMR-derived lipoprotein particle concentrations and sizes in overweight/obese African-American and white American women with pre-diabetes

Parameter	Groups	African-Americans	White Americans	p Value
N	110	69	41	
VLDL size (nm)	46.4±6.7	44.9±6.04	48.6±6.9	0.004
LDL size (nm)	20.1±0.51	21.1±0.47	20.7±0.54	0.01
HDL size (nm)	9.0±0.51	9.2±0.5	8.9±0.5	0.009
VLDL and chylomicron particles (total) (nmol/L)	46.4±26.8	38.9±24.8	59.2±25.6	<0.001
Large VLDL and chylomicron particles (nmol/L)	3.3±3.9	2.4±3.4	4.9±4.1	0.0007
Medium VLDL particles (nmol/L)	18.3±15	13.7±12.2	25.9±16.2	0.0001
Small VLDL particles (nmol/L)	24.9±14.1	22.9±13.9	28.3±13.8	0.05
Total LDL particles (nmol/L)	1234.3±349.5	1226.2±378.3	1248.2±286.5	0.732
IDL particles (nmol/L)	120.2±81.8	126.9±87.6	109±70.5	0.267
Large LDL particles (nmol/L)	538.2±190.1	560.5±197	500.8±176.1	0.113
Small LDL particles (nmol/L)	575.9±286.8	538.8±294.1	638.4±266	0.07
Total HDL particles (µmol/L)	33.6±5.7	33.9±5.08	33.1±5.4	0.47
Large HDL particles (µmol/L)	5.6±3.2	6.1±3.1	4.64±3.1	0.02
Medium HDL particles (µmol/L)	10.7±5.4	10.7±5.4	10.5±5.4	0.86
Small HDL particles (µmol/L)	17.9±17.	17.7±4.9	17.9±5.3	0.407

Values are mean±SD.

HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; VLDL, very low-density lipoprotein.

Furthermore, the large (2.4 ± 3.4 vs 4.9 ± 4 nmol/L, $p=0.007$), medium (13.7 ± 12.2 vs 25.9 ± 16.2 nmol/L, $p<0.001$) and small (22.9 ± 13.8 vs 28.3 ± 13.8 nmol/L, $p=0.05$) VLDL particle concentrations were significantly lower in AA versus WA women (table 4).

CORRELATION COEFFICIENTS

Insulin resistance has been associated with increased prevalence of CVD risk factors and incident CVD morbidity and mortality. Therefore, we examined the correlations between Si and the traditional and NMR-derived lipid and lipoprotein parameters in our overweight/obese AAW and WAW. Using Spearman's univariate regression, in the overall group, Si correlated with fasting insulin ($r=-0.342$, $p=0.0019$), 2-hour insulin ($r=-0.2147$, $p=0.0327$), HDL ($r=0.1867$, $p=0.089$) and large HDL particle concentration ($r=0.3092$, $p=0.0094$). However, Si did not correlate with the other covariates such as fasting and 2-hour glucose, triglycerides and LDL-C and LDL particles. Si correlated with large VLDL ($r=-0.2189$, $p=0.0454$). However, Si did not correlate with other NMR-derived lipoprotein particles. We used multiple regression models (robust regression models) to examine relationships between Si and fasting and 2-hour serum insulin and glucose after adjusting for age, BMI and WHR. These adjustments did not significantly change the relationships between Si and the other remaining lipids and lipoproteins as a group in our study.

DISCUSSION

The racial and ethnic differences in CVDs require a thorough understanding of the mediators of CVD risk factors, including lipid and lipoprotein metabolism. Traditionally measured LDL-C levels are positively and HDL-C levels are negatively associated with coronary heart disease in non-blacks,²⁻⁵ but it remains controversial in blacks.^{4-5 8 10 15-18} In contrast, NMR-derived HDL particles inversely correlated with incident coronary heart disease in all populations including blacks.^{2-5 15-18} Thus, in the present study, we investigated in detail these lipids/lipoproteins to explain the coronary heart disease paradoxes in AA and WA women with pre-diabetes. We found that fasting serum glucose, insulin and c peptide were lower in our overweight/obese AA compared with WA women. In addition, incremental serum glucose responses were lower in AA versus WA women with pre-diabetes. The lower glucose levels in AA occurred in the face of higher A1C values in AA than WA women. Our study confirmed that for similar glucose levels, AA women have higher A1C. We found that the incremental integrated insulin and c peptide responses did not differ in AA versus WA women. Our findings suggest that, despite the marked obesity, AA women in our study appear to be more insulin sensitive than WA women. This was confirmed by HOMA-IR. Surprisingly, Si index was not significantly different in

our AA versus WA women, perhaps due to the marked obesity. Thus, the current findings of glucose homeostasis in overweight/obese AA and WA women with pre-diabetes were somewhat different from our previous reports⁷⁻⁹ and those of others.^{10 11 14}

Abnormalities in lipids and lipoproteins are regarded as the pivotal mechanism(s) underlying atherosclerosis in humans.^{1-5 10} This concept is based partly on the lower HDL-C and/or high LDL-C atherogenic model that has led to the development of several potent anti-lipid agents for clinical use.²²⁻²⁵ However, there are inconsistencies in the relations between HDL-C and LDL-C and atherosclerosis in certain genetic and ethnic/racial populations.^{2-3 6 10 14 18-21 23-25} Another major risk factor for CVD is insulin resistance, which also varies among different ethnic/racial populations. The insulin resistance is generally associated with lower HDL-C and higher LDL-C levels in several populations. In this context, we have previously demonstrated that glucose-tolerant AA women are more insulin resistant than their white counterparts.⁷⁻⁹ But paradoxically, AA women with insulin resistance have either normal or higher HDL-C and concomitantly lower serum triglycerides using traditional methods or enzymatic assays than their white counterparts.⁷⁻¹¹ This was confirmed in the present study. Although the mechanism for this paradox remains unknown, differences in the hepatic lipoprotein synthesis and clearance have been implicated.^{11 20} In the present study, HDL-C and Apo A1, the largest protein of HDL particle, regarded as a potent anti-atherogenic protein, were higher in AA than WA women. However, this apparent CVD benefit of HDL-C and Apo A1 does not appear to occur in AA.

In order to explore HDL-C further, we measured the HDL particle concentrations and sizes in our overweight/obese AA and WA women with pre-diabetes. We found that HDL particle size was significantly higher in AA than WA women. Specifically, we found that the large HDL particle concentrations were higher in AA than WA women, but there were no significant differences in the small and medium HDL concentrations. However, we should note that HDL has several qualitative antiatherogenic properties. These include its anti-oxidant, anti-inflammatory, antithrombotic, as well as vasculodilatory and antiapoptotic activities.²⁶⁻³⁰ These properties have been attributed in part to different HDL particle sizes and concentrations. In this regard, the large HDL is functionally less active than the small particle size, but this remains debatable. In this context, it has become clear that the total concentration of HDL alone is inadequate to predict its antiatherogenic activity.^{13 16-19} Furthermore, previous studies report that the use of cholesteryl ester transfer protein (CETP) inhibitors (torcetrapid²⁴ and dalcetrapib)²⁵ resulted in markedly elevated HDL levels, but surprisingly, did not reduce incident coronary heart disease. Although the reasons are unclear, we have postulated that the HDL particles in these studies could have been dysfunctional.

In this context, Gaillard *et al*³¹ and Dodani *et al*³² have previously reported that there are racial/ethnic differences in HDL functionality. Indeed, we have shown that HDL is dysfunctional in non-diabetic, postmenopausal AA compared with WA women as assessed by paraoxonase 1 (PON1), which cosegregates with HDL in the circulation. We found that PON1, a potent anti-inflammatory and antioxidant, was 50% lower in non-diabetic, postmenopausal AA than WA women.³¹ In the current study, our AA women had higher concentrations of large HDL particles which are reported to have less antiatherogenic properties. The reasons for the higher levels of HDL-C and HDL remain uncertain. However, this is believed to be partly genetic as demonstrated in single nucleotide polymorphism (SNP) such as HDL_{Milano} and HDL_{Mendelian} traits.²¹ In this regard, Miljkovic-Gacic *et al*²⁰ reported higher frequency of hepatic lipase gene (LIPC-514C>T) polymorphism in blacks of African ancestry. This SNP has been associated with higher serum HDL-C and lower triglycerides in AA than in WA. We believe measuring HDL particle concentrations provides better assessment of CVD risks in blacks as shown in the Dallas Heart Study^{6 16} and the Jackson Heart Study,¹⁷ as well as the Multi-Ethnic Study of Atherosclerosis (MESA) trial.^{13 15}

Obesity is associated with insulin resistance, low HDL-C and high serum triglycerides when compared with normal weight individuals who are insulin sensitive in several racial/ethnic populations. The higher serum triglycerides have been attributed to increased hepatic VLDL synthesis and decreased peripheral clearance. However, because of the consistently lower serum triglycerides found in AA and other black populations residing in diverse geographic locations, we investigated the VLDL particle concentrations and sizes in our overweight/obese AA and WA women with pre-diabetes. We confirmed that fasting serum triglycerides measured by traditional enzymatic methods were significantly lower in AA than WA women with pre-diabetes. This observation provides a strong support for the mechanism of lowering fasting serum triglycerides in AA and blacks in the African diaspora.^{4 7-11} In this regard, Miljkovic-Gacic *et al*²⁰ studied lipoprotein subclasses and particle size differences in Afro-Caribbeans, African-Americans and white Americans. The findings were consistent with our report⁷⁻⁹ and those of other investigators.^{10 11}

LDL, the major carrier of circulating cholesterol, and Apo B100, which binds to the LDL receptors on the cell surface, have been extensively investigated over the past five decades. Indeed, LDL-C plays a dominant role in the pathogenesis of atherosclerosis. This observation has led to the development of pharmacological strategies to lower hepatic LDL synthesis (eg, statins, niacin, etc) with some positive CVD outcomes.^{13 22-25 32} To this end, modification of LDL particle by higher glucose, free oxygen radicals, impaired HDL function, low PON1, etc, results in higher levels of oxidized LDL, which is

associated with vascular tissue (endothelial) injury as well as subclinical inflammation and atherogenesis.^{26-30 32} Most importantly, LDL has been shown to circulate in several subclasses with varying atherogenic properties.^{12 13 15-19} In this context, the small, dense LDL particles (pattern B) are regarded as the most highly oxidizable (and hence the most atherogenic LDL particles) compared with the large, less dense and more buoyant LDL particles.^{10 15} In the current study, we found lower small LDL particles in overweight/obese AA versus WA women with pre-diabetes. We propose that this observation could partly explain the racial and ethnic differences in atherosclerosis in AA versus WA populations. Therefore, we investigated the differences in the subclasses of LDL particles in our overweight/obese AA and WA women with pre-diabetes. We found that the total LDL particle concentrations were similar in overweight/obese AA than WA women with pre-diabetes. However, the small LDL, but not the large and medium particle concentrations, was lower in AA versus WA women. Thus, the LDL profile in our AA should theoretically result in lower propensity for atherosclerosis in AA women. Unfortunately, this projected CVD outcome and benefits, paradoxically, do not occur in AA.^{4 10 12 13} Conversely, despite this favorable lipid/lipoprotein profile, AA women suffer two to four times higher rates of CVD outcomes than their white counterparts.¹⁻⁴

Strengths and limitations of the study

We found several strengths and some weaknesses in our study. First, the ability to examine NMR-derived lipoprotein particles in the biethnic population provided confirmation of some of the previous studies using traditional lipid and lipoprotein measurements. Second, we studied participants with pre-diabetes (not with diabetes), thus eliminating the confounding effects of severe hyperglycemia on lipid/lipoprotein metabolism and insulin resistance. Third, we confirmed that VLDL particles (large, medium and small) are major carriers of serum triglycerides. These were significantly lower in overweight/obese AA than WA women with pre-diabetes. This provides potential explanation for the lower serum triglycerides in AA than WA.

We observed some weaknesses in our study. In this regard, the most important limitation in our study was the small sample size. This is in contrast with the MESA trial,¹⁵ the Jackson Heart Study and the Framingham Offspring Cohort Study,¹⁷ which measured the NMR-derived lipoprotein particles and sizes in large samples of multiethnic populations residing in diverse geographic locations. Second, in the current study, AA women were over-represented and oversampled (1.5:1 ratio). However, we found no differences when AA and WA numbers were matched. Third, our study was on overweight/obese AA and WA women with pre-diabetes residing in one geographic location. Thus, we cannot extrapolate our results to lean participants, men or the general population. Fourth, we did not extensively

examine the role of social determinants of CVD, such as socioeconomic status, level of education, smoking, physical activity, etc in the current study. Finally, our study was cross-sectional; hence, cause-and-effect relationships of lipids/lipoproteins and CVD and its outcomes could not be ascertained.

In summary, overweight/obese AA women with pre-diabetes with insulin resistance had higher serum HDL-C and Apo A1 and lower serum triglycerides, which are theoretically considered favorable antiatherogenic profiles, compared with WA women. We believe these differences cannot be explained by macronutrient intake but deserve further elucidation. The NMR-derived lipoprotein particle profiles were also more favorable in overweight/obese AA women than WA women with pre-diabetes. We confirmed that the lower serum triglycerides previously reported in AA women can be attributed to lower VLDL levels in them than in WA women. The total HDL particle concentration was higher in AA women due to the presence of higher concentrations of large HDL, which is considered less cardioprotective. Finally, we found higher concentrations of large LDL, but lower small and medium LDL particles, which is considered a less atherogenic profile in our AA than WA women with pre-diabetes.

We conclude that NMR spectroscopy provided better lipoprotein profiles for atherogenesis than traditional measurements in overweight/obese AA compared with WA women with pre-diabetes. Nevertheless, because AA women suffer twofold to fourfold higher CVD mortality,¹⁻⁴ we speculate that the favorable lipids and lipoproteins derived using traditional and NMR methods in our study are unable to explain the paradoxically higher CVD morbidity and mortality previously found in AA women compared with WA women and warrant further investigations.

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