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Research Article

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Aims and Method. The present study examined the relationship between the metabolic risk profile (MRP) and total testosterone (TT) and free testosterone using the free androgen index (FAI) and sex hormone binding globulin (SHBG) in 36 Caucasian American (CA) and 30 African-American (AA) women volunteering for a weight loss study. *Results*. After controlling for age, significant relationships were found between TT and diastolic blood pressure ($P = .004$ and $P = .015$ in CA and AA women, resp.). Additionally, total cholesterol ($P = .003$), low density lipoprotein cholesterol ($P = .004$), apolipoprotein B ($P = .006$), and the total cholesterol/high density lipoprotein cholesterol ($P = .027$) were significantly related to TT in AA women only. In CA women, similar measures of glucose/insulin status related to FAI, were also related to SHBG. In both CA and AA women, SHBG was related to waist ($P = .031$ and $P = .022$ resp.). *Conclusion*. Our findings showed racial disparities in the relationship between the sex steroid milieu and the MRP in overweight/obese CA and AA women.

1. Introduction

In women, a more android fat distribution pattern signified by increased visceral adipose tissue (VAT) is associated with high testosterone and low-sex hormone binding globulin (SHBG), adverse lipids and lipoproteins, and altered glucose/insulin homeostasis [1, 2]. Since sex steroids and SHBG serve as a marker of androgen status, they may be considered a valuable marker of metabolic variables associated with cardiovascular disease (CVD) risk [3].

In a large study of both pre- and postmenopausal women, Bell and colleagues [4] confirmed the importance of SHBG as an independent predictor of the metabolic risk profile (MRP) in women across the lifespan. However, despite higher rates of CVD [5], obesity [6], and type II diabetes [7], few studies have targeted overweight or obese African-American (AA) women.

In premenopausal women in the SWAN study [8], investigators did compare racial groups and found a sig-

nificant correlation between SHBG, testosterone, and the MRP in minority women. Their results showed consistent hormone-risk factor relationships across racial groups that in some instances appeared stronger in Asian and Caucasian women. They also showed significant differences between testosterone and triglycerides (TG) in Black and Hispanic women. However, due to large BMI variances found among racial groups, investigators cautioned against comparing relationships across racial lines. In contrast, Berman and colleagues [9] found several significant relationships between SHBG and the MRP in postmenopausal Caucasian American (CA) women despite the fact that no significant relationships were evidenced in postmenopausal AA women. Unfortunately, investigators failed to control for intra-abdominal or VAT in either study, leaving potential mediating processes uninvestigated across racial groups.

In a study of premenopausal Hispanic and CA women by Haffner and colleagues [10, 11] a significant inverse relationship between SHBG, insulin resistance, and atherogenic

lipids was found to be similar across Hispanic and CA women supporting similarity in these relationships across racial lines. The similar and significant relationships observed in this study remained after controlling for BMI and VAT [10]. However, controlling for insulin negated a number of relationships that were previously significant, demonstrating the importance of insulin in modulating this relationship. In contrast, other investigations found that after controlling for VAT, neither SHBG nor testosterone were related to the MRP reaffirming the importance of VAT in modulating this relationship [1, 2].

Given the inconsistencies in the literature and lack of emphasis in overweight/obese women, the present study examined total testosterone (TT), free testosterone, using the free androgen index (FAI) and SHBG, in relation to MRP in a group of overweight/obese premenopausal CA and AA women. A secondary purpose was to examine whether these relationships were mediated by VAT or insulin in each group of women.

2. Subjects and Methods

2.1. Subjects. Participants were volunteers interested in a weight loss program. Subjects were required to be premenopausal, weight stable for a minimum of three months, possessing a BMI above 25, and free of known metabolic disease such as diabetes, hyperlipidemia, hypertension, polycystic ovary syndrome, or CVD. Subjects who were pregnant or lactating, amenorrheic, or taking medications or hormones that would affect blood pressure (BP), carbohydrate, or lipid metabolism were excluded from the study. All subjects were required to be eumenorrheic having a normal monthly menstrual cycle for the proceeding six months. A total of 66 subjects (36 CA; 30 AA) met the criteria necessary for participation in the study. One weekday and one weekend day were randomly selected for testing and evaluation of participants. A second consecutive weekday and weekend day were also provided to maximize participation of all subjects. Subjects gave informed consent and completed all testing procedures in accordance with the ethical standards and guidelines of the Institutional Review Board guidelines for human subjects at the University of Miami.

2.2. Physical and Anthropometric Measurements. Body weight was measured to the nearest 0.1 kg. Height was measured to the nearest 0.5 cm, and BMI was calculated as weight (kg) divided by the square of height (m²). Waist circumference was measured using a spring tension measuring tape (Gays Mills, WI) midway between the lower rib margin and iliac crest, [12]. All anthropometric measurements were performed by the same investigator who recorded the mean of the two measurements to the nearest 1.0 mm.

Following an overnight fast, systolic and diastolic blood pressure (SBP and DBP, resp.) were taken in one setting following a 5-min rest interval. Duplicate blood pressure measurements were taken from the left upper arm, averaged, and recorded to the nearest 2.0 mmHg.

2.3. Magnetic Resonance Imaging (MRI). The abdominal region was measured by MRI using a 1.5T instrument (Siemens Medical Systems, Iselin, NJ). Spin-echo imaging was performed using a T1-weighted sequence with a 147-ms repetition time and 4.8 ms echo time. Ten mm thick images with a 2.5-mm gap between images were obtained during a single breath hold sequence. A total of seven images were obtained in each subject with the central slice obtained at the L4-L5 intervertebral disk space. VAT was defined as adipose tissue contained within the boundaries of the rectus abdominis, internal obliques, quadratus lumborum, and long back muscles while subcutaneous adipose tissue (SAT) was adipose tissue located between the skin and the same group of muscles. Both VAT and SAT were computed by summing the respective VAT and SAT partial volumes (cm³) and converting to liters.

2.4. Serum Measurements. Blood was withdrawn from the antecubital vein after a 12-h fast and following centrifugation. Serum was analyzed within one week of withdrawal. All serum measurements were taken while subjects were seated quietly for at least five minutes. Total cholesterol [13], high-density lipoprotein cholesterol (HDL) [14], its subfractions [15], and *TG* [16] were measured by the Diabetes Research Institute Lipid Laboratory, University of Miami. Serum standards used for calibration were developed by the Diabetes Research Institute and calibrated against serum samples from the Centers for Disease Control and Prevention Laboratory, Atlanta, GA. Very low density lipoprotein cholesterol was estimated by TG divided by five, and low density lipoprotein cholesterol (LDL) was indirectly calculated by subtracting HDL and very low density lipoprotein cholesterol from total cholesterol [17].

The peak particle diameter for major LDL subfractions was determined on a gel scan based upon standards of known diameter as described by Krauss and Burke [18]. The LDL size of the predominant peak for a subject was identified as the subjects' LDL peak particle size (pps).

Apolipoprotein B in serum was measured by turbidimetric immunoassay using a commercially available kit (Incstar, Stillwater, MN) according to procedures outlined by the manufacturers. All apolipoprotein B procedures have been developed in accordance with the guidelines set forth by the International Federation of clinical chemistry.

Fasting glucose levels were determined spectrophotometrically at a wavelength of 340 nm using a hexokinase reaction developed by Roche (Roche Diagnostic System, Nutley, NJ). Serum insulin was measured by radioimmunoassay of serum using a Coat-A-Count insulin procedure (Diagnostic Products, Los Angeles, CA). The insulin resistance was assessed by using the homeostasis model assessment (HOMA) index, which divides the product of fasting insulin (μU/mL) and glucose (mmol/L) by 22.5 [19].

Serum SHBG was analyzed by immunoradiometric assay (Siemens Health Care Diagnostics; Los Angeles, CA). The TT was analyzed by Coat-A-Count radioimmunoassay using a testosterone-specific antibody (Siemens Health Care Diagnostics; Los Angeles, CA). Quality control procedures for

accuracy and reliability in the laboratory yielded intra-assay and interassay coefficients of variation of less than 5% for SHBG and less than 7.5% for TT. The FAI was the ratio of TT/SHBG in the same unit [20, 21].

2.5. Additional Assessments. Nutrient intake and alcohol consumption (g/day) were recorded using a three-day food log and analyzed using the Dine System (Buffalo, N.Y. 1994). Cigarettes smoked were determined using a medical history questionnaire. Subjects were administered the College Alumnus Questionnaire developed by Paffenbarger et al. [22] for evaluating daily physical activity. Education experience was measured using a 1–5 categorical scale modified from Rosmond et al. [23]. All subjects completed the Daily Stress Inventory which has been shown to be a significant correlate of endocrine measures of stress [24].

2.6. Statistical Analysis. All statistical analyses were completed using the Statistical Package for the Social Sciences, version 10.1 [25]. Means ± standard deviations of all variables were calculated for participants. Natural log transformation was used for TG, SHBG, and FAI while the square root of TT was used to achieve normality of distribution. Student's t-tests for unpaired samples were used to assess differences between racial groups. Since age showed a trend toward significant differences in CA and AA women, Pearson's correlation coefficients were performed after controlling for age, to examine the relationship between SHBG and sex steroids with the MRP in CA and AA women. These correlations were also conducted after controlling for VAT and insulin.

3. Results

3.1. Descriptives. A total of 66 subjects, 36 CA and 30 AA women, were evaluated for subject characteristics. A comparison between groups indicated that CA women had significantly higher VAT and TG levels than AA women ($P < .05$ for both). There was also a trend toward significant differences in waist circumference ($P = .058$), age ($P = .063$), and SHBG ($P = .073$). No other significant differences or trends in metabolic variables were noted between groups.

3.2. Association between Total Testosterone and the Metabolic Risk Profile. Pearson's correlation coefficient showed a significant inverse correlation between TT and BP ($P = .014$ and $P = .004$ for SBP and DBP, resp.) indicating that higher TT was associated with lower BP in CA women. This was observed concomitant with a positive relationship between SAT and TT ($P = .006$). In AA women, significant inverse relationships were also observed between TT and DBP ($P = .015$) as well as total cholesterol ($P = .003$), LDL ($P = .004$), Apo B ($P = .006$), and total cholesterol/HDL ($P = .027$). In both CA and AA women, all significant relationships remained, after controlling for VAT or insulin.

3.3. Association between Free Androgen Index and the Metabolic Risk Profile. Pearson correlation coefficient

showed significant positive relationships between FAI and waist circumference ($P = .048$), SAT ($P = .026$), glucose, insulin, and HOMA IR ($P \leq .01$ for all) and a negative relationship with HDL ($P = .043$) in CA women. Similar to what was observed for TT, significant relationships between FAI and DBP ($P = .018$), total cholesterol ($P = .009$), LDL ($P = .010$), and Apo B ($P = .029$) were found for AA women. Controlling for VAT had no impact upon aforementioned relationships in either CA or AA women. Controlling for insulin, however, negated all significant relationships between FAI and HOMA IR as well as serum lipid and lipoproteins in both CA and AA women.

3.4. Association between Sex Hormone Binding Globulin and the Metabolic Risk Profile. In CA women, SHBG was significantly and positively related to waist circumference ($P = .031$), VAT ($P = .004$), glucose ($P = .009$), insulin ($P = .001$), and HOMA IR ($P = .001$). In AA women, the only significant associations were observed between SHBG and waist circumference ($P = .022$). However, there was a trend toward significant relationships between SHBG and VAT ($P = .096$), insulin ($P = .056$), HOMA IR ($P = .090$), and HDL ($P = .094$) in AA women. Adjusting for insulin negated all significant correlations in both CA and AA women. Adjusting for VAT negated the significant relationship between SHBG and waist circumference in AA women.

4. Discussion

The present study revealed several interesting findings relevant to sex steroids, SHBG, and the MRP in CA and AA women. As expected, CA women showed higher VAT and TG levels than AA women, which had been reported in previous studies [26, 27]. After controlling for VAT, however, there were no longer significant differences in TG levels between groups indicating that lower TG in AA women were accounted for, in part, by their lower VAT levels. Controlling for VAT also eliminated any trends toward significance by group for waist circumference and SHBG, highlighting the importance of VAT in these relationships. In contrast, other studies have shown FAI to be significantly lower [8] and SHBG significantly higher [28] in Black compared to White subjects.

Interestingly, our findings showed an inverse relationship between TT and several metabolic variables observed in both CA and AA women (Table 2). The majority of research has suggested that women possessing higher androgens tend to display a more adverse MRP. Other studies, however, have shown no association between TT and the MRP [1, 2]. Still others have reported favorable associations between TT and metabolic variables [29, 30]. Equivocal results may be found because TT represents a combination of the more bioactive, free form as well as the inactive form bound with other proteins, that is, SHBG, albumin. In the present study, as TT went up, BP went down and this relationship was observed in both CA and AA women.

TABLE 1: Comparison of subject characteristics between CA and AA women using student's t-tests for unpaired samples.

Characteristics	CA women ($n = 36$)	AA women ($n = 30$)	P^a
Age (y)	41.94 ± 7.10	37.97 ± 9.94	.063
Height (m)	1.64 ± 0.07	1.62 ± 0.06	.168
Weight (kg)	94.79 ± 15.72	91.84 ± 14.03	.427
BMI (kg/m^2)	35.12 ± 5.35	34.88 ± 4.4	.850
Resting heart rate (beats/min)	73.67 ± 11.04	76.57 ± 10.90	.289
Stress[b]	67.71 ± 21.37	69.28 ± 31.64	.815
Education[c]	2.56 ± 0.50	2.30 ± 0.66	.077
Physical activity (kcal/wk)	$983.65 \pm 1,033.60$	892.88 ± 895.74	.723
Cigarettes (no./d)	2.11 ± 6.96	1.53 ± 5.10	.707
Alcohol	5.56 ± 13.78	2.27 ± 5.65	.226
Metabolic risk profile			
Waist (cm)	107.29 ± 14.50	101.03 ± 11.27	.058
WHR	0.86 ± 0.06	0.87 ± 0.08	.536
SAT (L)	4.68 ± 1.28	4.51 ± 1.40	.603
VAT (L)	**1.20 ± 0.48**	**0.76 ± 0.30**	$< .001$
Glucose (mmol/L)[d]	4.80 ± 0.57	4.76 ± 5.77	.758
Insulin (μU/mL)	14.84 ± 8.13	14.75 ± 5.77	.957
HOMA IR	3.13 ± 1.81	3.20 ± 1.39	.878
Systolic blood pressure (mmHg)	126.67 ± 14.89	123.00 ± 14.21	.313
Diastolic blood pressure (mmHg)	82.11 ± 10.13	82.20 ± 8.70	.970
Triglycerides (mg/dL)	**144.50 ± 78.44**	**107.77 ± 67.58**	**.048**
Total cholesterol (mmol/L)	5.34 ± 1.32	5.14 ± 1.41	.552
HDL (mmol/L)	1.44 ± 0.42	1.45 ± 0.29	.931
HDL$_2$ (mmol/L)	0.36 ± 0.23	0.41 ± 0.28	.428[e]
LDL (mmol/L)	3.10 ± 0.96	3.13 ± 1.25	.909
LDL pps (nmol/L)	27.11 ± 0.71	27.15 ± 0.57	.799
Apo B (mg/dL)	106.50 ± 29.22	102.70 ± 30.69	.609
Total cholesterol/HDL	3.91 ± 1.26	3.69 ± 1.35	.488
Lp(a) (mg/dL)	24.86 ± 20.60	36.85 ± 23.59	.093
SHBG and Sex Steroids			
SHBG (nmol/L)[e]	33.88 ± 22.98	43.80 ± 30.73	.073[e]
TT (ng/mL)[e]	0.27 ± 0.11	0.33 ± 0.19	.170[e]
FAI	4.24 ± 3.55	4.11 ± 4.83	.685[e]

Values are means $\pm$ SD. SAT indicates volume of subcutaneous adipose tissue; VAT indicates volume of visceral adipose tissue; IR, insulin resistance calculated as (insulin, μU/mL $\times$ glucose, mmol/L) $\div$ 22.5; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; LDL pps-low-density lipoprotein, peak particle size; Apo B, apolipoprotein B; SHBG, sex hormone binding globulin; TT, total testosterone, FAI, free androgen index, calculated as TT (ng/mL) $\times$ 3.467 $\times$ 100/SHBG (nmol/L).
[a]Values obtained from a Student's t-test for unpaired samples.
[b]Graded numerically, with the higher number indicating a higher stress level.
[c]Graded on a scale of 1 to 5, with the higher number indicating a higher level of education.
[d]One outlier was excluded from the analysis.
[e]t-test performed on transformed values.

Paradoxically TT has been reported to have both vasodilatory and vasoconstrictive effects [31]. According to Livingstone and Collison [32], there may be a "physiological window" within which normal androgen/estrogen levels confer health benefits and beyond which may show detrimental effects. Based upon the literature, our women demonstrated low normal levels of TT. If estrogen levels fell along the same lines, a more balanced androgen/estrogen ratio would have translated into a more favorable association between TT and the MRP.

Unexpected was the significant inverse relationship between TT and several lipoproteins in AA women. Total cholesterol/HDL is considered one of the most powerful predictors of CVD [33] and in AA women, Apo B, another marker of atherogenic risk, shared almost 28% of its variance with TT. Thus, at a given level of TT, AA women demonstrated lower levels of total cholesterol, LDL, and ApoB than CA women. This contrasts with other studies using minority groups [8, 34] which showed very little if any association between TT and the MRP. In the Swan study [8], TT was

TABLE 2: Pearson's correlation coefficients between total testosterone (square root) and the metabolic risk profile after adjusting for age.

Metabolic risk profile	Caucasian American women (r value)	P value	African American women (r value)	P value
BMI (kg/m²)	0.124	.478	−0.072	.711
WAIST (cm)	0.235	.174	−0.117	.547
SAT (L)	**0.452**	**.006**	−0.104	.593
VAT (L)	0.100	.567	0.176	.361
Glucose (mmol/L)	0.220	.211	−0.02	.924
Insulin (uμ/mL)	0.281	.108	0.004	.986
HOMA IR	0.246	.161	0.008	.967
SBP (mmHg)	**−0.411**	**.014**	−0.298	.116
DBP (mmHg)	**−0.481**	**.004**	**−0.448**	**.015**
TG (mg/dL)	0.015	.934	−0.175	.394
Total cholesterol (mmol/L)	−0.259	.140	**−0.555**	**.003**
HDL (mmol/L)	−0.242	.168	−0.058	.778
HDL$_2$ (mmol/L)	−0.025	.888	−0.267	.187
LDL (mmol/L)	−0.320	.065	**−0.550**	**.004**
LDL pps (nmol/L)	−0.029	.871	0.238	.241
Apo B (mg/dL)	−0.239	.173	**−0.528**	**.006**
Total cholesterol/HDL-C	−0.101	.571	**−0.433**	**.027**
Lp (a)	−0.082	.654	−0.008	.967

SBP, systolic blood pressure, DBP, diastolic blood pressure, SAT, subcutaneous adipose tissue volume, VAT, visceral adipose tissue volume, HOMA IR, insulin resistance calculated as (insulin × glucose) ÷ 22.5, HDL, high density lipoprotein cholesterol, HDL$_2$, high-density lipoprotein subfraction 2, LDL, low-density lipoprotein cholesterol, Apo B, apolipoprotein B, LDL pps, low density lipoprotein peak particle size, TG, triglycerides, log transformed, BMI, body mass index, WAIST, waist circumference.

correlated with TG accounting for only a modest difference by race in this variable. Interestingly, the incidence of the Metabolic Syndrome [35] and of coronary heart disease [36] is only slightly greater in AA than CA women despite AA women having much greater rates of overweight/obesity. This, however, may be more closely related to differences in other risk factors such as high blood pressure and diabetes which tend to be higher in AA women.

Tchernof and Després [3] have maintained that VAT plays an important role in moderating the relationship between sex steroids and coronary risk factors. However, when we controlled *for* VAT, all significant correlations with *TT* remained. They also remained when we controlled for insulin. Thus, the mechanisms responsible for the significant relationship found in AA women require further study. Past research shows no consensus opinion regarding the relationship between endogenous steroids and serum lipids or lipoproteins [3]. Our finding of a significant relationship in AA women adds to the controversy surrounding this issue.

As expected, significant and positive relationships were observed between FAI and insulin/glucose status, as well as central obesity measures (SAT and waist) [1, 2, 10, 11]. In AA women, a different case scenario was observed. Similar to that shown with TT, FAI was significantly related to DBP and atherogenic lipoproteins (total cholesterol, LDL, and Apo B) but not measures of glucose/insulin status.

Upon further evaluation, CA compared to AA women showed roughly double the magnitude of correlation between FAI and either fasting insulin or HOMA IR. Furthermore, glucose shared almost 20% of its variance with FAI in CA women, while in AA women, there was virtually no shared variance between glucose and FAI. This discrepancy accounted for an FAI/glucose relationship ten times greater in CA than AA women.

Other studies [37, 38] have suggested that AA women are more insulin resistant than CA women even after adjustment for VAT [37]. In postmenopausal women, Ryan and colleagues [37] have shown a 60% lower glucose uptake concomitant with a higher insulin level in AA compared to CA women. Although there were no significant differences between fasting insulin or HOMA IR in CA and AA women, our data reaffirms racial differences in the relationship between FAI and the MRP specifically with regard to the FAI/glucose relationship. This is particularly relevant since diabetes risk is 2.4 times greater in AA than CA women [7]. Further research is warranted to support the mechanisms responsible for these differences.

Our work contrasted with the findings of Pedersen et al. [2] who did not find significant relationships between FAI and the MRP, independent of VAT. The only exception was the relationship between HDL and FAI. In fact, our results showed that insulin played a more critical role than

TABLE 3: Pearson correlation coefficients between free androgen index (log transformed) and the metabolic risk profile after adjusting for age.

Metabolic risk profile	Caucasian American women (r value)	P value	African American women (r value)	P value
BMI (kg/m^2)	0.188	.279	0.149	.440
WAIST (cm)	**0.337**	**.048**	0.323	.087
SAT (L)	**0.376**	**.026**	−0.080	.682
VAT (L)	0.308	.072	0.316	.095
Glucose (mmol/L)	**0.519**	**.002**	−0.044	.829
Insulin (uμ/mL)	**0.436**	**.0100**	0.264	.192
HOMA IR (ratio)	**0.478**	**.004**	0.236	.247
SBP (mmHg)	−0.197	.257	−0.301	.112
DBP (mmHg)	−0.211	.223	**−0.435**	**.018**
TG (mg/dL)	0.195	.269	−0.067	.746
Total cholesterol (mmol/L)	−0.196	.269	**−0.504**	**.009**
HDL (mmol/L)	**−0.349**	**.043**	−0.249	.220
HDL$_2$ (mmol/L)	−0.211	.231	−0.238	.243
LDL (mmol/L)	−0.253	.149	**−0.499**	**.010**
LDL pps (nmol/L)	−0.092	.598	−0.028	.891
Apo B (mg/dL)	−0.151	.192	**−0.428**	**.029**
Total cholesterol/HDL-C	0.035	.844	−0.297	.141
Lp (a)	−0.097	.594	−0.125	.542

BMI, body mass index, WAIST, waist circumference, SAT, subcutaneous adipose tissue volume, VAT, visceral adipose tissue volume, SBP systolic blood pressure, DBP, diastolic blood pressure, HOMA IR, insulin resistance calculated as (insulin × glucose) ÷ 22.5; HDL, high-density lipoprotein cholesterol, HDL$_2$, high density lipoprotein subfraction 2, LDL, low density lipoprotein cholesterol, LDL pps, low-density lipoprotein peak particle size, Apo B, apolipoprotein B, TG, triglycerides, log transformed.

VAT in modulating these relationships. Upon controlling for insulin, the relationship between FAI and all lipid variables in both CA and AA women was negated. These findings are supported by Haffner and colleagues [11, 39] who proposed that insulin plays a more primary role in relation to the sex steroid milieu. Insulin is reported to stimulate androgen production in the ovary, and bioavailable androgens may be more closely associated with insulin and insulin resistance [11, 40]. Androgens also play a role in modulating hepatic lipase activity [2] which would explain subsequent changes in the relationship between FAI and HDL, as well as TG after controlling for insulin. It does not explain how insulin modulates the relationship between FAI and other lipids such as total cholesterol, LDL, or APO B, shown in AA women.

In CA women, the same four variables related to FAI were also related to SHBG (waist, glucose, insulin, and HOMA IR). Relationships were additionally observed between SHBG and VAT. These relationships confirm the deleterious effects of low SHBG/high FAI reported in many previous studies [2, 4, 8, 10]. Furthermore, insulin has been shown to suppress hepatocellular SHBG production [41] and both insulin and VAT have been reported to impact the relationship between SHBG and the MRP [1, 2, 10]. Controlling for insulin, but not VAT, negated all significant relationships between SHBG and the MRP in CA women. This indicates the importance of insulin in modulating relationships between SHBG and variables associated with CVD risk.

In AA women, the only significant relationship observed with SHBG was waist circumference. At first glance our findings mirror the results of those by Berman et al. [9] who found significant relationships between SHBG and the MRP in overweight/obese postmenopausal CA but not AA women. They surmised that differences were related to a higher postheparin lipase activity and a lower level of hepatic lipase in AA women which would explain a greater capacity to clear TG's from circulation in their AA women. Our study showed a lower VAT which was related to lower TG in AA women. Thus, the mechanism proposed for racial differences in their postmenstrual women may not be applicable to premenopausal women in our study.

Upon further examination, however, there was a trend toward significance between SHBG and insulin as well as HOMA IR, HDL, and VAT in AA women (see Table 4). Although these correlations were somewhat lower in AA than CA women, they do not negate the fact that some clinical associations may be occurring in AA women. In the SWAN study [8] and the study by Sherif and colleagues [34], they did find significant relationships between SHBG and HDL in AA women, which were both lower than that of AA women in the present study. This is likely due to the much larger number of subjects in the SWAN study and slightly higher number of AA subjects in the study by Sherif [34]. Subjects in the SWAN study [8] also showed considerably lower BMI levels than our study. Certainly, a

TABLE 4: Pearson correlation coefficients between sex hormone binding globulin (log transformed) and the metabolic risk profile after adjusting for age.

Metabolic risk profile	Caucasian American women (r value)	P value	African American women (r value)	P value
BMI (kg/m^2)	−0.267	.121	−0.171	.375
WAIST (cm)	**−0.365**	**.031**	**−0.424**	**.022**
SAT (L)	−0.230	.184	0.045	.817
VAT (L)	**−0.478**	**.004**	−0.315	.096
Glucose (mmol/L)	**−0.442**	**.009**	−0.029	.89
Insulin (uμ/mL)	**−0.527**	**.001**	−0.379	.056
HOMA IR (ratio)	**−0.542**	**.001**	−0.339	.090
SBP (mmHg)	−0.191	.271	0.183	.341
DBP (mmHg)	−0.183	.800	0.257	.179
TG (mg/dL)	−0.331	.056	−0.137	.504
Total cholesterol (mmol/L)	−0.040	.820	−0.192	.373
HDL (mmol/L)	0.262	.135	0.335	.094
HDL$_2$ (mmol/L)	0.300	.085	0.100	.627
LDL (mmol/L)	0.117	.511	0.182	.373
LDL pps (nmol/L)	0.164	.355	−0.233	.252
Apo B (mg/dL)	−0.013	.943	−0.079	.700
Total cholesterol/HDL	−0.111	.533	−0.021	.917
Lp (a)	0.071	.696	−0.202	.322

BMI, body mass index, WAIST, waist circumference, SAT, subcutaneous adipose tissue volume, VAT, visceral adipose tissue volume, SBP systolic blood pressure, DBP, diastolic blood pressure, HOMA IR, insulin resistance calculated as (insulin × glucose) ÷ 22.5; HDL, high-density lipoprotein cholesterol, HDL$_2$, high-density lipoprotein subfraction 2, LDL, low-density lipoprotein cholesterol, LDL pps, low-density lipoprotein peak particle size, Apo B, apolipoprotein B, TG, triglycerides, log transformed.

higher number of AA women in our study would have been helpful.

It remains unclear as to why some of the aforementioned relationships, particularly with regard to glucose/insulin status, were considerably stronger in CA than AA women. Investigators in the SWAN study [8] concluded that relationships were, in general, similar across racial lines being stronger in certain racial groups and weaker in others. With regard to the relationship between SHBG and the MRP, our results tended to reinforce their findings.

There were, however, several limitations noted with respect to this study. First, all relationships were based upon a single blood draw at one time point. It would have been better to obtain multiple samples particularly in a cross-sectional study. Furthermore, an n of 66 subjects which included a biracial group of women, limited our power. Since this was a cross sectional study, it was difficult to determine any temporal or causal relationships between sex steroids, SHBG, and the MRP. We could only speculate primary relationships based upon the strength of the correlation analysis before and after controlling for potential confounders, that is, VAT and insulin. Since many variables were correlated with each other, it was difficult to determine if some relationships were operating independently or via their association with other metabolic variables. Although women were recruited from the same medical institution and consisted of nurses, nurse assistants, and technicians,

it would have been helpful to examine the socioeconomic status of CA and AA women. A few women in both groups were in their mid to late 40's and may have been perimenopausal. This transition period prior to menopause may be linked to a progressive reduction in ovarian function, reduced estrogen levels, and stable androgen levels. Estrogen measurements would have been helpful. Although age was controlled for in all analysis, this may have affected the androgen/estrogen balance in several women. Furthermore, blood sampling was done randomly and not at uniform times in the menstrual cycle. Since total and LDL cholesterol show variability across follicular and luteal phases of the cycle [41], it may have affected relationships or lack thereof between the sex steroid milieu and serum lipoproteins in CA and AA women. It would have been preferable to sample all women at the same time in their menstrual cycle; however, it is hoped that the random selection of testing dates and blood withdrawal will have prevented any undue influences in our results. Finally, free testosterone was not measured directly but computed indirectly as FAI using both TT and SHBG in the formulation. Although FAI is considered a viable correlate of free testosterone, the direct measurement of free testosterone would have been preferable, particularly in women, in the low normal range. Although investigators sampled first thing in the morning following an overnight fast to reduce variability in androgen levels, it is always preferable to measure free testosterone

directly. Fortunately other confounding variables such as physical activity, smoking, alcohol, and stress known to affect the MRP were not found to be significantly different between groups.

5. Conclusions

Given aforementioned limitations, several important findings were noted. First, independent of VAT or insulin, TT showed several significant and cardioprotective relationships with several variables in the MRP, in both CA and AA women. Second, our study reinforced racial differences in the relationship between androgens and the MRP. This was evidenced by the significant relationships found between TT and FAI and serum lipoproteins in AA women that were absent in CA women. There were also significant relationships found between FAI and glucose/insulin status in CA women that were absent in AA women. Furthermore, the aforementioned relationships were not mediated by VAT but were more strongly influenced by insulin. Racial differences were also noted with respect to the strength of the relationship observed between SHBG and the MRP. These tended to be stronger in CA women. Future research is recommended to further elucidate the mechanisms responsible for these contrasting relationships and trends in premenopausal CA and AA women.

Disclosure

None of the authors reported any disclosures and there is no conflict of interest that would prejudice the impartiality of the research reported.

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