Sex Hormone–Binding Globulin and Insulin Resistance in African-American Women

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Sex hormone–binding globulin (SHBG) binds testosterone, determining the level of free, biologically active hormone, and is a sensitive indicator of androgen status in women. SHBG is strongly correlated with high-density lipoprotein (HDL), central obesity, and insulin sensitivity in Caucasian and Mexican-American women, thereby acting as a biologic marker for cardiovascular disease risk. The purpose of this study was to determine if SHBG was a significant correlate of metabolic cardiovascular risk factors in African-American women. Eighty-one nondiabetic, normotensive African-American women were enrolled (mean age, 30 years). After excluding women on oral contraceptives (n = 19), 62 women were examined during the follicular phase of the menstrual cycle. All subjects underwent an oral glucose tolerance test (OGTT) and a euglycemic-hyperinsulinemic insulin clamp, and the lipid and sex hormone levels were measured. Correlation analyses showed a significant correlation between SHBG and the following variables in women: central obesity, body mass index (BMI), HDL cholesterol, apolipoprotein B (apoB), insulin sensitivity adjusted for lean mass (M'), and the sum of insulin during the OGTT. The strongest correlates of SHBG in women were measures of insulin resistance ($r = .421$, $P < .001$). SHBG appears to be a biologic marker for insulin resistance, which is linked to cardiovascular risk, in African-American women.

IN WOMEN, SEX HORMONES play an important role in the development of cardiovascular disease. Epidemiologic studies have reported that a decline in ovarian steroidogenesis is associated with an increase in cardiovascular risk in postmenopausal women.[1] The Nurses' Health Study found that oral estrogen replacement therapy (ERT) in postmenopausal women is associated with a 50% reduction in cardiovascular mortality.[2,3] In randomized clinical trials, ERT is associated with an improvement in markers for cardiovascular disease, including plasma lipids and fibrinogen.[4] Exogenous testosterone negates the beneficial effects of estrogen.[5] There is also some evidence suggesting that in women the relative androgen/estrogen balance is a stronger correlate than either total testosterone or total estradiol with the metabolic risk factors for cardiovascular disease.[6-9]

Sex hormone–binding globulin (SHBG) is an indirect measure of the testosterone to estrogen ratio and has been used as a marker for androgen status. SHBG is a circulating steroid-binding protein produced by the liver that is analogous to androgen-binding protein produced by the testes. SHBG binds reversibly and with high affinity to testosterone.[10] The unbound (free) fraction of testosterone is the biologically active form. SHBG was originally described as a reservoir that regulates the amount of free hormone.[11] SHBG has also been postulated to interact with target-tissue surface receptors to determine selective tissue uptake.[12] SHBG is the most important determinant of the plasma distribution of testosterone in women and is a sensitive in vivo index of both free and SHBG-bound testosterone levels.

In a large population study of Mexican-American and Caucasian women, Haffner et al[13-15] did not detect an association of total testosterone or total estradiol with cardiovascular risk. However, in both premenopausal and postmenopausal women, there was an association of low plasma SHBG with insulin resistance and an atherogenic lipid profile.[13-15] Other investigators have found significant negative correlations between SHBG and plasma insulin, glucose, and triglyceride concentrations in Caucasians.[16-18] These studies also report a significant positive correlation of SHBG with high-density lipoprotein (HDL).[18,19]

These reports indicate that in premenopausal Caucasian and Mexican-American women, low SHBG and increased androgen are associated with insulin resistance and an atherogenic lipid profile. An androgenic biochemical profile is also linked with non–insulin-dependent diabetes mellitus (NIDDM) in Mexican-American and Caucasian women.[6,16] Despite high rates of cardiovascular disease and NIDDM in African-American women, there are few data on androgen balance relative to the risk for these diseases.

The purpose of this study was to determine if there is an association between SHBG and cardiovascular risk factors, such as insulin resistance and low HDL, in premenopausal African-American women. We hypothesized that African-American women with low serum SHBG would have higher free testosterone, lower HDL, and greater insulin resistance compared with women with high serum SHBG.

SUBJECTS AND METHODS

Population

The study was conducted with clinically well, nondiabetic African-Americans. Each participant was drawn from a cohort that has been under study in investigations of blood pressure and cardiovascular risk factors. Participants enrolled in this study included normotensives (blood pressure <135 mm Hg systolic and <85 mm Hg diastolic) and borderline hypertensives (blood pressure ≥135 and <150 mm Hg systolic or ≥85 and <96 mm Hg diastolic), based on repeated measurements of blood pressure.

Enrollment assessment consisted of physical examination, anthropometric measurements (height, weight, skinfold thickness, and circumference of the arm, hips, thigh, and waist), and blood pressure determination. Causal systolic (first phase) and diastolic (fifth phase) blood pressure measurements were obtained by auscultation with a mercury-column sphygmomanometer with subjects in the seated position

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following a 10-minute rest period. The average of two determinations was used as the blood pressure at the time of the metabolic evaluation. From the anthropometric measurements, percent body fat and fat-free mass were calculated.[20]

After an introductory interview, each subject returned to the clinical research unit for an oral glucose tolerance test (OGTT), which was scheduled in the morning following a 12-hour fast. A fasting blood sample for serum lipids and glucose was obtained, and then a 75-g glucose solution (Glucola; Ames Laboratories, Elkhart, IN) was ingested. Blood samples were obtained at 30, 60, 90, and 120 minutes following ingestion of the glucose load, and were assayed for glucose and insulin concentrations. The serum sample was sent to the Lipid Research Laboratory of the Hospital of the Medical College of Pennsylvania, where total cholesterol, HDL cholesterol, and total triglycerides were analyzed using standard enzymatic methods and an automated analyzer (model 704; Hitachi, Tokyo, Japan). HDL was isolated according to the method of Bachorik et al.[21] Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation.[22] Apolipoprotein A1 (apoA), apolipoprotein B (apoB), and lipoprotein(a) [Lp(a)] were assayed turbidimetrically using commercial antibodies (Boehringer-Mannheim, Mannheim, Germany).

All women had regular menstrual cycles (28- to 33-day interval) and were studied during the follicular phase of the menstrual cycle. Sex hormone levels were determined following an overnight fast. The plasma SHBG level was measured by an immunoradiometric assay with a commercially available kit (DELFIA; Wallace, Turku, Finland). Plasma free-testosterone and estradiol levels were measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Laboratory quality-control procedures for reliability and precision resulted in intraassay and interassay coefficients of variation of less than 5% for all radioimmunoassays, including SHBG and free testosterone.

The subjects returned for a third time for an insulin clamp study. The euglycemic-hyperinsulinemic clamp to measure insulin-stimulated glucose utilization (M) was performed according to methods previously described.[23] Insulin-stimulated glucose utilization was computed as the mean glucose infusion rate during the final 60 minutes of hyperinsulinemia and expressed as milligrams per kilogram per minute (M). Using anthropometric measures, fat-free mass was calculated for each subject, and insulin-stimulated glucose utilization was also expressed as milligrams per kilogram fat-free mass per minute (M′). Plasma glucose was determined with the glucose oxidase technique (Glucostat, YSI model 27; YSI, Yellow Springs, OH). Plasma insulin was determined with a solid-phase radioimmunoassay (Coat-A-Count; Diagnostic Products). Coefficients of variation for interassay and intraassay variability for glucose, insulin, and lipid assays were less than 5%.

Data Analysis

Bivariate correlations among numerically continuous variables were examined using Pearson correlation coefficients. Instead of using a repeated-measures ANOVA for OGTT data, we used the sum of insulin levels during the OGTT as the parameter in the continuous data analysis. Differences in mean values were considered statistically significant for P less than .05. Stepwise multiple linear regressions were used to examine multiple correlations among variables, and to build a regression model for SHBG by the other variables. Based on a theoretical model that variables in several categories will significantly determine both M and M′, a stepwise multiple linear regression analysis was used to determine the model of best fit for M and M′ by independent variables in several categories.

All 17 variables of Table 3 were considered as independent variables in a stepwise multiple linear regression analysis to produce a regression model of SHBG on the best linear combination of the other measures. The stepwise computer algorithm for the regression equation selects at the first step the single highest correlated variable with the dependent variable. At the second step, the algoritm selects the variable that produces the highest canonical correlation based on two independent variables with the dependent variable. Therefore, variables that are highly correlated with the first independent variable entered usually are not entered into the regression. The computer algorithm continues until there are no additional statistically significant ($P < .05$) increases in the prediction of SHBG on the best linear combination of independent variables. From the data in this study, there are some highly correlated independent variables, such as weight and body mass index (BMI). The algorithms are not disrupted or negated by this multicolinearity. When one set of highly correlated parameters is entered into the model, and it is usually the strongest correlate with the dependent variable, there is no additional predictive value for the others.

Table 1. Serum SHBG and Free-Testosterone Levels in the Women

Subjects	No.	SHBG (nmol/L)	Free-Testosterone (nmol/L)
Total group	81	45.8 ± 25.0	1.35 ± 1.17
Oral contraceptive nonusers	62	39.2 ± 21.0*	1.45 ± 1.19
Oral contraceptive users	19	68.8 ± 24.8	0.99 ± 1.02

*$P < .001$ *v* oral contraceptive users.

RESULTS

A total of 81 subjects were enrolled and had complete data sets available for analysis. Table 1 lists the mean SHBG and sex hormone levels. As expected, the women had high SHBG and low free testosterone. Oral contraceptive users (n = 19) had a significantly greater mean SHBG compared with nonusers. Because of the significant difference in SHBG levels between oral contraceptive users and nonusers, the users were excluded, with a final sample of 62 women for data analysis.

The demographic data for the study sample of young African-American women unexposed to exogenous steroids are provided in Table 2. The mean BMI of 30.9 kg/m^2 exceeds Kumanyika's criteria for obesity in women (BMI ≥ 27.8).[24]

In a series of correlation analyses, the relationship of SHBG with cardiovascular risk parameters was examined. The results of bivariate correlation analyses of SHBG with blood pressure and anthropometric variables are listed in Table 3. SHBG was negatively correlated with BMI ($r = -.27$, $P = .027$) and percent body fat ($r = -.39$, $P = .003$).

For SHBG and lipids (Table 3), SHBG was significantly positively correlated with HDL ($r = .31$, $P = .013$) and negatively correlated with apoB ($r = -.28$, $P = .024$) and triglycerides ($r = -.27$, $P = .031$). No statistically significant relationships between SHBG and LDL, apoA, and Lp(a) were detected.

There was a statistically significant correlation of SHBG with

Table 2. Demographics of Study Population

No. of subjects	62
Ethnicity	African-American
Age (yr)	31.4 ± 3.4
Weight (kg)	82.5 ± 22.9
BMI (kg/m^2)	30.9 ± 8.3
Systolic blood pressure (mm Hg)	118.4 ± 12.6
Diastolic blood pressure (mm Hg)	75.7 ± 11.1
Body fat (%)	33.7 ± 6.4
Central body fat index	1.12 ± 0.38

NOTE. Central body fat index = subscapular/triceps skinfold thickness.

Table 3. Correlations of SHBG With Risk Parameters in African-American Women

Risk Parameter	Pearson *r*	*P*
Blood pressure and anthropometric measurements		
Weight (kg)	−.28	.03
BMI (kg/m²)	−.27	.03
Systolic blood pressure (mm Hg)	−.08	.51
Central body fat index	−.22	.07
Percent body fat	−.39	.003
Lipids (mg/dL)		
Total cholesterol	−.03	.85
HDL cholesterol	.31	.01
LDL cholesterol	−.10	.42
Triglycerides	−.27	.03
ApoA	.19	.12
ApoB	−.28	.02
Lp (a)	−.18	.16
Measures of insulin resistance		
Total M	.48	.001
M′	.45	.001
Σ insulin	−.26	.04
I/G 120 min	.44	.001
Glucose 0 min	−.25	.05

NOTE. Central body fat index = subscapular/triceps skinfold thickness.

Abbreviations: M, insulin sensitivity; M′, insulin sensitivity corrected for fat-free mass; Σ insulin, sum of insulin in OGTT; I/G 120 min, insulin to glucose ratio at 120 minutes in OGTT; glucose 0 min, fasting glucose in OGTT.

measures of insulin sensitivity. Figure 1 depicts the positive correlation of SHBG with the measured insulin sensitivity corrected for body fat (M′) ($r = .45$, $P < .001$). In addition, there was a significant negative correlation of SHBG with the sum of insulin during the OGTT ($r = -.26$, $P = .039$). Overall, the data analysis detected a consistent strong relationship of SHBG with the parameters of insulin resistance (Table 3).

Using all 62 cases in a stepwise multiple linear regression analysis, we found that M′ was the only statistically significant correlate of SHBG. The results are summarized in Table 4. We

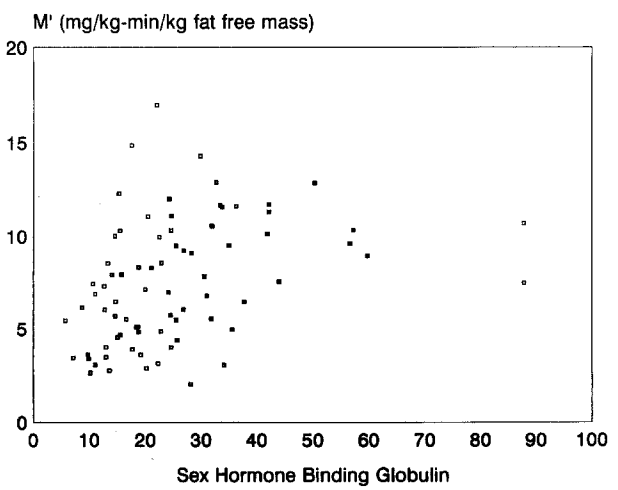

Fig 1. Correlation of SHBG with insulin sensitivity corrected for body fat (M′). Pearson $r = .45$, $P < .001$, N = 62.

Table 4. Multiple Linear Regression Models of SHBG (N = 62)

Variable	*R*	Overall F Ratio	*P*	Slope	SE of Slope	*t* Statistic
Total M	.62	11.6	.001	3.43	0.94	3.66
I/G at 120 min				−26.99	7.31	−3.69
Sum of insulin				0.06	9.15	2.94

found that the total M (insulin sensitivity), the insulin to glucose ratio at 120 minutes during the OGTT, and the sum of insulin values during the OGTT were the three statistically significant variables in the multiple linear model with SHBG. The analyses indicate that the major determinants of SHBG are parameters of insulin resistance.

DISCUSSION

In a sample of young African-American premenopausal women, we found significant correlations between SHBG and a number of cardiovascular risk factors. The bivariate correlations were strongest among measures of insulin resistance and weaker among lipid parameters and anthropometric measures. The multiple linear regression analyses examining multivariate associations among all of the parameters tested with SHBG showed that measures of insulin resistance are the strongest correlates of SHBG.

SHBG synthesis is modulated by sex hormones. The binding of sex hormones with plasma proteins, including SHBG, determines tissue uptake and subsequent activity of sex hormones in vivo. SHBG levels are higher in women compared with men[25] and higher in children compared with adults, and decrease at puberty in both sexes.[26,27] Women with clinical androgen excess, including those with polycystic ovarian syndrome (PCOS) and idiopathic hirsutism (IH), have low SHBG compared with unaffected women.[28,29] Our sample excluded women with known PCOS or IH. None of the women reported here had clinical signs of PCOS, and all had regular menses. Data from our sample suggest that women with relative androgen excess who do not meet criteria for syndromes of androgen excess can also have lower levels of SHBG, indicating that androgen excess is relative and can be a continuous and a dichotomous variable.

Factors other than androgen excess are known to alter SHBG levels. SHBG is elevated in patients with anorexia nervosa and returns to normal levels with weight gain.[30] SHBG is also strongly linked with obesity, with lower SHBG levels documented in obese women.[31] Not all obesity is associated with low SHBG: central obesity, rather than peripheral obesity, is associated with a reduction in SHBG. Obese adolescent girls with a waist to hip ratio (WHR) greater than .86 have significantly lower SHBG and higher free and total testosterone compared with obese girls with a WHR less than .80.[32] Bernasconi et al[31] reported a significant negative correlation between SHBG and BMI in a sample of hirsute Caucasian women, and this relationship was strongest in women with upper-body obesity. Approximately half of the young African-American women in our study were obese, and SHBG was negatively associated with BMI and percent body fat. However, the multiple linear regression analysis detected the direct measurement of insulin

resistance as the leading determinant of SHBG in the model, which suggests that the relationship of obesity with SHBG is secondary to insulin resistance in this population. These data are consistent with reports on other ethnic groups.[13]

Obesity is known to be a condition of hyperinsulinemia. The evidence that insulin modulates SHBG includes in vitro studies showing that insulin inhibits hepatic SHBG synthesis.[33] While both obesity and upper-body adiposity are also associated with decreased SHBG, Haffner et al[13] have shown that the significant negative correlation of insulin sensitivity with SHBG persists following adjustment for BMI and measures of upper-body adiposity. These data raise the possibility that insulin resistance may be the condition that contributes to lower SHBG. While insulin resistance may be a prediabetic phase, the association of SHBG with diabetes has also been described. SHBG is low in adolescent diabetics and in adults with NIDDM.[34] SHBG is significantly lower in young female diabetics compared with nondiabetic controls.[35] In longitudinal studies, low SHBG has been used to predict the development of NIDDM in women.[6,16] Our cross-sectional sample excluded diabetics. A prospective follow-up study of this group will be needed to determine if low SHBG is predictive of NIDDM. However, over 25% of the living mothers of these subjects are diabetic.

Several reports have described a significant positive relationship between SHBG and HDL cholesterol. These investigators have verified a correlation of SHBG with HDL that was independent of obesity.[6-9,36] However, whether the correlation of HDL with SHBG was independent of insulin resistance was not mentioned. We have previously reported the significant correlation of insulin resistance with HDL in this young African-American population.[37] Again in this study, the relationship was present. Thus, it is possible that SHBG is one part of a complex interrelationship among sex hormones, obesity, and insulin metabolism in women.

SHBG is a marker for androgen status in women, and Nestler et al[38] reported cosegregation of relative androgen excess with metabolic risk factors for cardiovascular disease in women with PCOS. There is also evidence that SHBG levels can be modified. For example, exogenous estrogen therapy results in an increase of SHBG.[10] In a recent investigation by Velazquez et al,[39] women with PCOS received metformin therapy to increase insulin sensitivity. Concurrent with the increase in insulin sensitivity was an increase in SHBG levels. In another study, women with PCOS were given either a diet low in calories or a diet high in calories. Both diets were identical in fat content. Women who received the hypocaloric diet lost weight and also had an increase in the SHBG level. Both metformin therapy and weight reduction[40] increase insulin sensitivity. The increase in SHBG observed in both treatments may be an indirect effect and related to the change in insulin sensitivity. Interventions directed at improving insulin sensitivity in African-American women will be necessary to determine if SHBG and/or other risk factors can be altered.

Our study is the first to show that SHBG is significantly related to risk factors for cardiovascular disease in African-American women. Data from our study indicate that SHBG correlates directly with insulin sensitivity corrected for fat-free mass and with HDL cholesterol, and negatively with BMI, percent body fat, and plasma insulin. These data are consistent with population studies of Caucasian and Mexican-American women. SHBG is a significant correlate of insulin resistance in African-American women. Greater androgenicity, as reflected by low SHBG, may be a phenotype that predicts cardiovascular disease in African-American women.

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