

Sex Hormone–Binding Globulin and the Free Androgen Index Are Related to Cardiovascular Risk Factors in Multiethnic Premenopausal and Perimenopausal Women Enrolled in the Study of Women Across the Nation (SWAN)

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Background—Recent clinical trials have shifted attention away from estrogens and toward androgens and sex hormone–binding globulin (SHBG) as potential mediators of increasing cardiovascular (CV) risk in women at midlife.

Methods and Results—The correlation between reproductive hormones and CV risk factors was evaluated in a multiethnic (white, black, Hispanic, Chinese, and Japanese) sample of 3297 premenopausal and perimenopausal women. Testosterone and estradiol (E_2) were evaluated along with SHBG and the free androgen index (FAI), the amount of testosterone not bound by SHBG. Low SHBG and high FAI were strongly and consistently related to elevated CV risk factors (higher insulin, glucose, and hemostatic and inflammatory markers and adverse lipids) even after controlling for body mass index ($P < 0.001$ for all). Low levels of E_2 were associated with elevated CV risk factors to a lesser degree. These observations were consistent across the 5 ethnic groups. Compared with whites, blacks had higher levels of SHBG and lower levels of FAI, and Chinese had lower levels of SHBG and higher levels of FAI.

Conclusions—Low SHBG and high FAI are strongly associated with CV risk factors in racially diverse women, and thus, androgens likely play a role in the CV risk profile of perimenopausal women. (*Circulation*. 2005;111:1242-1249.)

Key Words: hormones ■ aging ■ sex ■ menopause ■ risk factors

Women are known to suffer coronary heart disease 10 to 20 years later than men,¹ leading to the longstanding hypothesis that endogenous ovarian hormones offer a protective effect on the development of coronary heart disease. Conversely, recent clinical trials have shown that hormone replacement therapy does not reduce the risk of coronary disease in postmenopausal women,^{2,3} suggesting that declining estrogen levels are not the key determinant of accelerated cardiovascular disease (CVD) risk associated with the menopausal transition.

Androgens and sex hormone–binding globulin (SHBG) have been linked to adverse CV risk factors in both premenopausal and postmenopausal women,^{4,5} with increased testosterone⁴ and decreased SHBG^{4,5} strongly associated with central adiposity, increased triglycerides, and decreased HDL cholesterol levels. Low levels of SHBG have also consistently been linked to higher rates of diabetes.^{6,7} We have

recently shown that the association between hemostatic factors and insulin resistance is strongest in the presence of low SHBG.⁸ Recently, SHBG and the free androgen index (FAI) have also been linked with CVD events, primarily through an increase in CV risk factors.⁹ Thus, androgens and SHBG are reemerging as potential mediators of CV risk in women at midlife.^{9–11}

Most of the research linking low SHBG and high androgens to CV risk has been restricted to white women and has focused on women who have reached postmenopause. The purpose of the present report is to look broadly at the association between reproductive hormones and CV risk factors in 3297 premenopausal and early perimenopausal women enrolled in the Study of Women’s Health Across the Nation (SWAN), an ethnically diverse cohort including white, black, Chinese-American, Japanese-American, and Hispanic women.

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Methods

Participants

Data from the baseline visit of the SWAN were used for the present analyses. SWAN is a multicenter, multiethnic, longitudinal study designed to characterize the biological and psychosocial changes that occur during the menopausal transition in a community-based sample. Details of the study design and recruitment have been previously published.¹² In brief, SWAN is being conducted at 7 sites: Boston, Mass; Chicago, Ill; the Detroit, Mich, area; Los Angeles, Calif; Newark, NJ; Pittsburgh, Pa; and Oakland, Calif. A total of 3302 women aged 42 to 52 years were enrolled from 1996 to 1997. Of these, 1550 are white, 935 black, 286 Hispanic, 250 Chinese, and 281 Japanese. The Hispanic group includes those of Central American, Mexican, and Caribbean origin.

At the time of enrollment, women had an intact uterus and at least one ovary and were not pregnant or breast-feeding. All participants were still menstruating, and women who used oral contraceptives or hormone replacement in the prior 3 months were excluded. Thus, all baseline data were for women exposed to only endogenous hormone levels. Women were classified as early perimenopausal if they reported a prior increase in cycle variability. The institutional review boards of the participating institutions approved this study, and all women signed informed consent forms before participation.

Blood Assays

A fasting blood sample was targeted to the follicular phase of the menstrual cycle (days 2 to 5). All samples were maintained at 4°C until separated and then were frozen at -80°C and shipped on dry ice to a central laboratory. Standard CV risk factors were assayed at Medical Research Laboratories (Lexington, Ky), which is certified by the National Heart, Lung, and Blood Institute, Centers for Disease Control and Prevention Part III program.¹³ Lipid and lipoprotein fractions were analyzed in EDTA-treated plasma. Total cholesterol and triglycerides were analyzed by enzymatic methods. HDL cholesterol was isolated after addition of heparin and 2 mol/L MnCl₂. Serum insulin was measured by a radioimmunoassay (DPC Coat-a-count) procedure and monitored as part of the monthly quality assurance program by the Diabetes Diagnostic Laboratory at the University of Missouri. Glucose was measured with a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics). The HOMA insulin resistance index was calculated from fasting insulin and glucose as (fasting insulin × fasting glucose)/22.5.¹⁴

Tissue-type plasminogen activator antigen (tPA) was measured in plasma with a double antibody in an ELISA (Imubind tPA ELISA, American Diagnostica). The assay uses human single-chain tPA as a standard calibrated against an international standard (NIBSAC). Plasma plasminogen activator inhibitor 1 (PAI-1) was measured by a sandwich procedure with a solid-phase monoclonal antibody and an enzyme-labeled goat second antiserum for detection (Imubind plasma PAI-1 ELISA, American Diagnostica). Fibrinogen and factor VII activity were measured in frozen, citrated plasma on an MLA Electra 1400C (Medical Laboratory Automation Inc) with a turbidometric detection system. C-reactive protein was quantified with an ultrasensitive rate-immunonephelometric method (hs-CRP on a BN 100, Dade-Behring).

Hormone assays were conducted at the University of Michigan SWAN Endocrine Laboratory with an ACS-180 automated analyzer (Bayer Diagnostics Corp). Serum follicle-stimulating hormone (FSH) concentrations were measured with a 2-site chemiluminescent immunoassay. SHBG was measured by a competitive chemiluminescent assay. Serum estradiol (E₂) concentrations were measured with a modified, offline ACS-180 (E₂-6) immunoassay. Testosterone concentrations were evaluated with the ACS-180 total testosterone assay modified to increase precision in the low ranges. The FAI was used estimate the amount of testosterone unbound by SHBG and thus, immediately biologically active. FAI was calculated as 100 × T / (28.84 × SHBG). This results in the amount of free testosterone in nanomoles per liter.

Physical Measures

Blood pressure was measured in the right arm with the participant seated after at least 5 minutes of rest. Three sequential blood pressure values were taken, and the final 2 were averaged. Height and weight were measured without shoes with participants wearing light clothing. Portable scales were calibrated weekly, and stationary clinic devices were calibrated monthly. Waist circumference was measured over undergarments or light clothing. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

Women were considered to have the metabolic syndrome if they met at least 3 of the following 5 criteria: triglycerides ≥150 mg/dL; HDL <50 mg/dL; blood pressure ≥130 mm Hg systolic, ≥85 mm Hg diastolic, or on hypertensive medication; fasting glucose ≥110 mg/dL and/or diabetes; and waist circumference ≥80 cm for Chinese and Japanese women and ≥88 cm for white, Hispanic, and black women.

Statistical Methods

All statistical procedures were performed with the maximum number of available observations for each given CV risk factor. Smoking status was defined as never, former, and current smoking. Alcohol intake was categorized into a 3-level variable, consisting of non-drinkers, drinkers below the median kilocalories of alcohol consumed per day, and drinkers at or above the median kilocalories of alcohol consumed per day. Cycle day of blood draw was dichotomized as within days 2 through 5 of the menstrual cycle or outside days 2 through 5 of the menstrual cycle. This second category included observations for which cycle day was unknown.

Numerous variables were not normally distributed, and thus, nonparametric tests were used wherever possible. Median values and interquartile ranges were calculated to describe the study sample. To assess associations between each hormone and covariate, partial Spearman correlation coefficients were calculated, after adjusting for age, ethnicity, study center, cycle day of blood draw, smoking status, alcohol intake, and BMI. Linear regression was used to evaluate race-by-hormone interaction terms. In these analyses, the natural logarithm of PAI-1, HDL, triglycerides, FSH, E₂, and FAI and the square root of testosterone and SHBG was used. Because of high collinearity among the CV risk factors, it was possible that any observed correlations between SHBG and FAI and multiple CV risk factors was being driven by just one or two risk factors. This possibility was explored by using stepwise multivariable regression, with forced inclusion of the covariates just listed, and transformed hormone variables as the response. Additional variables considered in the multivariable model statement were HDL, triglycerides, PAI-1, waist circumference, glucose, insulin, and HOMA insulin resistance index. These variables had the strongest partial correlation coefficients within groupings of similar variables (lipids/lipoproteins, inflammatory/hemostatic, glucose metabolism). Model fit was assessed with model residual plots.

Because of the high number of comparisons in this analysis, we considered a probability value of 0.01 to be statistically significant. For ethnicity-specific analyses, probability values <0.05 are presented because of the smaller sample size, but these should be considered with caution. All analyses were accomplished with SAS version 8.0 for Windows.¹⁵

Results

The 3302 women enrolled in SWAN included 935 blacks, 281 Japanese, 250 Chinese, 286 Hispanics, and 1550 whites. The number of women with nonmissing data ranged from 3080 to 3297, depending on the CV risk factor being assessed (Table 1). The mean age of the women was 46.2 years, and 47% had reached early perimenopause, whereas the remaining 53% were classified as premenopausal. The CV risk factor profile for the full group and by ethnicity is presented

TABLE 1. Characteristics of the Study Sample

Variable	Full Sample							<i>P</i> for Ethnic Differences
	No.	Median (Interquartile Range)	Whites Median (Interquartile Range)	Blacks Median (Interquartile Range)	Hispanics Median (Interquartile Range)	Chinese Median (Interquartile Range)	Japanese Median (Interquartile Range)	
Age, y	3297	46.2 (4.2)	46.1 (4.3)	46.1 (4.2)	46.0 (3.7)	46.6 (3.9)	46.7 (4.3)	0.104
Body composition								
Waist circumference, cm	3247	83.0 (21.8)	82.0 (21.3)	91.9 (22.2)	86.0 (18.0)	76.0 (12.5)	71.8 (11.5)	<0.001
Body weight, kg	3269	70.5 (25.8)	70.6 (23.2)	82.0 (27.5)	69.9 (17.6)	56.1 (11.6)	54.6 (11.2)	<0.001
BMI, kg/m ²	3242	26.6 (9.2)	26.1 (8.4)	30.2 (10.0)	28.3 (6.7)	22.4 (4.0)	22.1 (4.2)	<0.001
Lipids/lipoproteins								
Total cholesterol, mg/dL	3279	192.0 (44.0)	192.0 (42.0)	190.0 (49.0)	195.0 (47.0)	189.5 (37.0)	195.0 (38.0)	0.360
Triglyceride, mg/dL	3116	91.0 (65.0)	91.0 (68.0)	85.0 (52.0)	117.0 (84.0)	96.0 (62.0)	94.0 (69.0)	<0.001
LDL, mg/dL	3080	114.0 (40.0)	114.0 (40.0)	116.0 (42.0)	120.0 (39.5)	105.0 (36.0)	112.0 (39.0)	<0.001
HDL, mg/dL	3279	54.0 (18.0)	54.0 (18.0)	53.0 (18.0)	50.0 (15.0)	59.5 (19.0)	60.0 (18.0)	<0.001
Lp(a), mg/dL	3223	18.0 (38.0)	12.0 (29.0)	40.0 (51.0)	19.0 (36.0)	11.0 (18.0)	13.0 (19.0)	<0.001
Inflammatory/hemostatic								
Factor VIIIc, %	3150	115.0 (36.0)	116.0 (37.0)	114.0 (36.0)	119.0 (43.0)	112.0 (27.0)	115.0 (30.0)	<0.001
Fibrinogen, mg/dL	3200	282.0 (90.0)	282.0 (81.0)	306.0 (99.0)	282.0 (80.0)	269.0 (55.0)	246.0 (63.0)	<0.001
PAI-1, ng/mL	3130	20.7 (22.1)	19.6 (22.0)	22.5 (23.6)	28.6 (22.5)	17.5 (17.6)	16.9 (16.5)	<0.001
tPA antigen, ng/mL	3151	7.3 (4.4)	7.0 (4.3)	8.4 (4.7)	8.9 (4.9)	5.9 (3.7)	6.5 (3.1)	<0.001
CRP, mg/L	3245	1.6 (4.1)	1.5 (3.6)	3.2 (6.7)	2.3 (4.2)	0.7 (1.3)	0.5 (0.9)	<0.001
Glucose metabolism								
Insulin, μ U/mL	3109	8.6 (7.1)	7.8 (6.2)	10.8 (8.8)	12.6 (11.6)	7.4 (4.0)	6.7 (3.8)	<0.001
Glucose, mg/dL	3119	91.0 (13.0)	90.0 (12.0)	93.0 (16.0)	92.0 (18.0)	92.0 (9.0)	91.0 (10.0)	<0.001
HOMA insulin resistance index	3109	1.9 (1.9)	1.7 (1.5)	2.6 (2.6)	2.8 (3.2)	1.7 (1.0)	1.5 (0.9)	<0.001
Blood pressure								
Systolic blood pressure, mm Hg	3295	115.0 (20.0)	112.0 (17.0)	123.0 (27.0)	120.0 (14.0)	110.0 (20.0)	109.0 (16.0)	<0.001
Diastolic blood pressure, mm Hg	3292	75.0 (12.0)	73.0 (12.0)	77.0 (16.0)	80.0 (9.0)	72.0 (13.0)	74.0 (11.0)	<0.001
Hormones								
FSH, mIU/mL	3292	15.9 (15.6)	15.3 (14.6)	16.6 (17.0)	15.6 (18.5)	16.5 (16.9)	14.5 (13.6)	0.230
Testosterone, ng/dL	3292	41.5 (26.5)	42.8 (26.6)	41.8 (27.4)	37.3 (24.2)	40.0 (23.2)	38.1 (24.4)	<0.001
E ₂ , pg/mL	3293	55.2 (55.7)	56.5 (54.7)	54.8 (54.4)	59.0 (70.3)	48.5 (53.7)	51.8 (54.0)	0.014
SHBG, nmol/L	3292	41.0 (29.5)	40.9 (29.3)	41.9 (27.9)	36.7 (26.1)	38.5 (27.5)	43.5 (38.2)	0.026
FAI	3292	3.6 (3.9)	3.8 (4.1)	3.6 (3.7)	3.3 (3.4)	3.7 (3.6)	3.3 (3.7)	0.005

Lp(a) indicates lipoprotein(a); CRP, C-reactive protein.

in Table 1 along with the baseline values of FSH, testosterone, E₂, SHBG, and FAI.

We have previously reported that the CV risk factors varied substantially by race, with black and Hispanic women having the highest total CVD risk.¹⁶ We have also previously reported that E₂ levels did not vary by ethnicity at baseline in SWAN but that FSH levels were higher and testosterone levels lower in both black and Hispanic women compared with whites.¹⁷

Spearman correlations between CV risk factors and hormone values are presented in Table 2. These values were adjusted for age, ethnicity, study center, cycle day of blood draw, smoking status, alcohol intake, and BMI. Higher FSH values were weakly correlated with lower BMI, higher

cholesterol, and lower insulin and insulin resistance values. In general, higher testosterone and lower E₂ values were associated with elevated CV risk factors. Although the correlations were in opposite directions, both testosterone and E₂ were significantly associated with BMI, triglycerides, PAI-1, and tPA; these correlations, however, were generally modest in magnitude. In addition, testosterone alone was weakly correlated with blood pressure and glucose, and E₂ alone was negatively correlated with waist circumference and LDL and positively correlated with HDL.

The most striking associations with CV risk factors occurred for SHBG and the FAI. Both a low SHBG and a high FAI were strongly and consistently related to adverse CV risk factors. Even after adjusting for BMI, low SHBG and high

TABLE 2. Spearman Correlation Coefficients Between Baseline CV Risk Factors and Hormone Levels, Adjusted for Age, Ethnicity, Study Center, Cycle Day of Blood Draw, Smoking, Alcohol Intake, and BMI

	FSH	Testosterone	E ₂	SHBG	FAI
Body composition					
Waist	-0.02	-0.01	-0.06†	-0.13‡	0.08‡
BMI*	-0.08‡	0.12‡	-0.08‡	-0.32‡	0.30‡
Lipids/lipoproteins					
Total cholesterol	0.10‡	0.04	-0.04	-0.06‡	0.07‡
Triglycerides	-0.02	0.08‡	-0.08‡	-0.21‡	0.19‡
LDL	0.10‡	0.01	-0.05†	-0.06‡	0.04
HDL	0.04	0.02	0.10‡	0.19‡	-0.11‡
Lp(a)	0.01	0.02	0.03	0.08‡	-0.04
Inflammatory/hemostatic					
Factor VII	0.02	-0.02	-0.02	-0.09‡	0.05†
Fibrinogen	-0.01	0.03	0.01	0.02	0.001
PAI-1	-0.02	0.08‡	-0.08‡	-0.29‡	0.26‡
tPA	-0.02	0.16‡	-0.06†	-0.16‡	0.21‡
CRP	-0.03	-0.01	-0.02	-0.08‡	0.03
Glucose metabolism					
Insulin	-0.06†	0.01	-0.004	-0.13‡	0.09‡
Glucose	-0.02	0.07‡	-0.04	-0.12‡	0.13‡
HOMA insulin resistance index	-0.06‡	0.02	-0.01	-0.14‡	0.12‡
Blood pressure					
Systolic	0.01	0.03	-0.01	0.001	0.02
Diastolic	0.01	0.07‡	-0.03	-0.04	0.06‡

See the footnote to Table 1 and text for explanation of abbreviations.

*Not adjusted for BMI.

†*P*<0.01; ‡*P*<0.001.

FAI were associated with elevated risk for every CV risk factor (Table 2). SHBG was most strongly associated with PAI-1, triglycerides, and HDL, and FAI was most strongly associated with PAI-1, tPA, triglycerides, and glucose.

In addition to the risk factors presented in Table 2, we evaluated the association of hormone factors with the presence or absence of diabetes. There were 194 women with diabetes. These women had higher testosterone, lower SHBG, and higher FAI values than women without diabetes (*P*<0.001 for all). There was no difference in FSH or E₂ values between the women with and without diabetes.

To determine whether the hormone–CV risk factor associations observed for the full group were consistent across ethnic groups, analyses were stratified by ethnicity (Table 3). In general, FSH values were not significantly related to CV risk factors within ethnic groups. The only exception to this was a negative correlation between FSH and BMI for whites and blacks only. For the other hormone values, ethnicity-specific results were similar to the results for the full group. Significant race-by-hormone interactions were found for each of the risk factors listed in Table 3, and these interactions primarily involved SHBG and FAI. In general, hormone–risk factor associations were in the same direction for all ethnicities, but the strength of the association varied. For example, the association of both low SHBG and high FAI with BMI was significant for all ethnicities but was particularly strong

among the Japanese and white women. Likewise, the association between low SHBG and high PAI-1 was substantial for all ethnicities but was particularly strong for the Chinese and Japanese women. Chinese and Japanese woman also had particularly strong associations between SHBG and HDL. Finally, among Chinese women, the association of both low SHBG and high FAI with waist circumference was particularly strong. The only risk factor for which the direction of the hormone association differed by ethnicity was triglycerides. Testosterone was positively associated with triglycerides for blacks and negatively associated for Hispanics.

Because obesity (BMI>30) was of particular concern as a confounding variable, we also stratified the analyses in Table 2 by obesity and tested for hormone-by-obesity interactions. The associations between hormone factors and CV risk factors were similar between obese and nonobese women. The only significant interaction was an FSH-by-obesity interaction for waist circumference (*P*<0.001). For the stratified analyses, the Spearman correlation between FSH and waist circumference was -0.03 among the nonobese women and -0.10 among the obese women.

Because many of the CV risk factors are highly correlated with one another, it is possible that many of the risk factor associations seen with SHBG and FAI are operating through one or two primary risk factors. To determine which CV risk factors contributed independent information to lower SHBG

TABLE 3. Spearman Correlations of Risk Factors With Hormones by Ethnicity, Adjusted for Age, BMI, Study Center, Smoking, Alcohol Use, and Cycle Day of Blood Draw

	Testosterone	E ₂	SHBG	FAI
BMI				
White	0.15‡	-0.13‡	-0.41‡	0.38‡
Black	0.11†	-0.05	-0.28‡	0.27‡
Hispanic	0.15*	-0.03	-0.27‡	0.30‡
Chinese	0.05	-0.15*	-0.23‡	0.21†
Japanese	0.16†	-0.12	-0.45‡	0.38‡
<i>P</i> for race×hormone interaction	0.880	0.330	0.002	0.011
Waist circumference				
White	-0.03	-0.06*	-0.13‡	0.08†
Black	0.02	-0.08*	-0.07*	0.04
Hispanic	0.03	-0.02	-0.19†	0.13*
Chinese	0.11	0.04	-0.21†	0.24‡
Japanese	-0.07	-0.04	-0.10	0.00
<i>P</i> for race×hormone interaction	0.699	0.845	<0.001	0.030
tPA				
White	0.14‡	-0.05*	-0.18‡	0.22‡
Black	0.20‡	-0.06	-0.09*	0.17‡
Hispanic	0.16*	-0.21†	-0.25‡	0.30‡
Chinese	0.20†	0.00	-0.15*	0.23‡
Japanese	0.13*	-0.02	-0.16*	0.19†
<i>P</i> for race×hormone interaction	0.911	0.564	0.003	0.269
PAI-1				
White	0.09‡	-0.08†	-0.29‡	0.27‡
Black	0.09*	-0.05	-0.19‡	0.20‡
Hispanic	0.09	-0.11	-0.26‡	0.25‡
Chinese	-0.07	-0.01	-0.41‡	0.29‡
Japanese	0.06	-0.10	-0.42‡	0.34‡
<i>P</i> for race×hormone interaction	0.278	0.701	<0.001	0.113
HDL				
White	0.03	0.13‡	0.21‡	0.13‡
Black	-0.01	0.06	0.14‡	-0.07
Hispanic	0.08	0.04	0.12	-0.04
Chinese	-0.03	0.04	0.24‡	-0.18†
Japanese	0.04	0.09	0.23‡	-0.13*
<i>P</i> for race×hormone interaction	0.810	0.207	0.017	0.070
Triglycerides				
White	0.04	-0.04	-0.20‡	0.15‡
Black	0.20‡	-0.07*	-0.13‡	0.22‡
Hispanic	-0.19†	-0.13*	-0.25‡	0.06
Chinese	0.09	0.02	-0.11	0.14*
Japanese	0.11	-0.15*	-0.22‡	0.22‡
<i>P</i> for race×hormone interaction	<0.001	0.381	<0.001	0.065

See the footnote to Table 1 and text for explanation of abbreviations.

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

and higher FAI, the square root of SHBG and log(FAI) were separately modeled by linear regression (Table 4). Age, site, ethnicity, cycle day of blood draw, smoking, and alcohol intake were included in each model. Risk factors that were

significantly and separately related to lower SHBG levels were greater BMI, higher triglycerides, lower HDL, higher PAI-1, and ethnicity ($P < 0.01$ for all). Blacks had higher levels of SHBG compared with whites, whereas Chinese had significantly lower levels of SHBG compared with whites. These same risk factors were related to FAI, with the addition of tPA and glucose. Blacks and Hispanics had significantly lower levels of FAI compared with whites, and Chinese had significantly higher levels of FAI compared with whites. Interestingly, for both SHBG and FAI, neither HOMA nor the presence of diabetes added further predictive value over and above the variables in the final model. When the analysis was restricted to the 2701 women without a history of diabetes, the results were similar, except that HDL and glucose were no longer related to FAI. The same was true when women with a history of the metabolic syndrome were also excluded (Table 4).

Discussion

These data suggest that among premenopausal and early perimenopausal women, hormones, particularly low SHBG and high FAI, are related to elevated CV risk factors. This observation was consistent across the 5 ethnic groups included in our population sample. Although hormone-risk factor associations were consistently present across ethnic groups, these associations were significantly stronger among some ethnic groups, often the Chinese and Japanese. These findings were consistent whether or not women with diabetes and/or the metabolic syndrome were included in the analysis.

Previous data have shown SHBG to be positively associated with HDL¹⁸ and physical fitness¹⁹ and negatively associated with obesity,^{7,18,20} upward fat distribution,²¹ triglycerides,²² insulin resistance,^{21,23,24} and diabetes.^{6,7} The data presented here are consistent with these findings and extend them to a racially diverse population sample. In addition, we have shown that SHBG and FAI are correlated with hemostatic factors, particularly PAI-1 and tPA. We have previously reported in this population sample that both low SHBG levels and high PAI-1 levels are related to greater insulin resistance and that the association between fibrinolytic markers and insulin resistance is strongest when SHBG is also low.⁸

The present study provides the unique opportunity to compare SHBG and FAI values across race. When data were adjusted for BMI and other CV risk factors, blacks had significantly higher SHBG and lower FAI values than did whites, and Chinese had significantly lower SHBG and higher FAI values than did whites. The association of SHBG and FAI with risk factors was consistent across racial groups. However, there is evidence that these associations are particularly strong for certain ethnic groups, most often Japanese and Chinese women. Racial differences in BMI within the SWAN population sample have been presented and discussed previously.¹⁷ It should be noted that the majority of the Japanese and Chinese women were in the lower tertile of the BMI distribution, whereas the majority of Hispanic and black women were in the upper tertile of the BMI distribution. This may be part of the underlying reason for some of the race-by-hormone interactions observed. The lower preva-

TABLE 4. CV Risk Factors Associated With SHBG and FAI Based on Linear Regression Modeling

	Full Sample		History of Diabetes Excluded		History of Diabetes or Metabolic Syndrome Excluded	
	Standardized β	<i>P</i>	Standardized β	<i>P</i>	Standardized β	<i>P</i>
Transformed SHBG*	n=2864		n=2700		n=2250	
BMI	-0.20	<0.001	-0.19	<0.001	-0.18	<0.001
HDL	0.18	<0.001	0.16	<0.001	0.14	<0.001
Triglycerides	-0.06	0.003	-0.07	<0.001	-0.05	0.012
PAI-1	-0.17	<0.001	-0.17	<0.001	-0.21	<0.001
Race (vs white)						
Black	0.08	<0.001	0.08	<0.001	0.07	0.003
Hispanic	0.04	0.159	0.05	0.104	0.03	0.307
Chinese	-0.09	<0.001	-0.09	<0.001	-0.11	<0.001
Japanese	-0.04	0.099	-0.04	0.116	-0.03	0.288
Transformed FAI*	n=2836		n=2672		n=2228	
BMI	0.17	<0.001	0.18	<0.001	0.18	<0.001
HDL	-0.06	0.004
Triglycerides	0.05	0.007	0.09	<0.001	0.08	<0.001
PAI-1	0.13	<0.001	0.14	<0.001	0.17	<0.001
tPA	0.14	<0.001	0.12	<0.001	0.10	<0.001
Glucose	0.05	0.010
Race (vs white)						
Black	-0.13	<0.001	-0.13	<0.001	-0.12	<0.001
Hispanic	-0.08	0.010	-0.08	0.010	-0.06	0.08
Chinese	0.06	0.017	0.07	0.014	0.07	0.014
Japanese	0.02	0.347	0.02	0.377	0.01	0.861

See the footnote to Table 1 and text for explanation of abbreviations.

*Models also controlled for age, site, smoking, alcohol intake, and cycle day of blood draw.

lence of obesity among the Chinese and Japanese women may have allowed the association between hormones and other risk factors to be more clearly observed in these groups. Though speculative, another possible explanation may relate to the observation that Chinese and Japanese women maintain relatively high levels of adrenal androgens. We have previously reported higher levels of dehydroepiandrosterone sulfate (DHEAS) early in the menopausal transition among Chinese and Japanese women relative to the other ethnic groups in SWAN.²⁵ Although DHEAS is a weak androgen, it can be converted peripherally to more bioactive compounds. It is possible, therefore, that Japanese and Chinese women have higher levels of total circulating bioactive androgens.

There are a number of potential mechanisms for the strong association between SHBG and CV risk factors. The positive correlation between HDL and SHBG is thought to be due to the influence of SHBG on the metabolism and/or production of HDL,⁵ although the precise mechanism is unknown. Insulin has a direct inhibitory effect on SHBG,^{26,27} and SHBG may thus be a marker of insulin resistance and/or the metabolic syndrome. We found that low SHBG was more highly associated with higher weight, triglycerides, and PAI-1 and low HDL than with elevated insulin or insulin resistance. This is consistent with the fact that SHBG has been found to predict the development of type 2 diabetes in

women, over and above the effects of glucose and insulin concentrations.⁶ Likewise, PAI-1 has also been shown to predict type 2 diabetes after controlling for insulin resistance.²⁸ Thus, the constellation of higher weight, triglycerides, and PAI-1 and lower HDL and SHBG may mark a process separate from but related to insulin resistance.

SHBG may be linked to CV risk factors through its association with androgens. Because SHBG binds preferentially to androgens,²⁹ a decrease in SHBG has a greater impact on the amount of circulating free androgens than free estrogens, and SHBG has been viewed as an index of androgenicity.⁶ High androgen levels are associated with alterations in glucose metabolism, an adverse lipid profile and higher blood pressure,^{30,31} higher rates of subclinical atherosclerosis,^{31,32} higher levels of coronary atherosclerosis,³⁰ and higher CV event rates.³⁰ Testosterone specifically has been linked to coronary atherosclerosis on angiography and was found to be more predictive of atherosclerosis than total cholesterol in postmenopausal women.³³ In the data presented here, higher levels of free testosterone were correlated with risk factors in a similar fashion to low levels of SHBG. Because the FAI is calculated with SHBG, it is difficult to determine which of these is the variable more closely linked to CV risk. A direct measure of free testosterone would be more useful in determining the relative effect of these 2 factors on CV risk.

Finally, it is possible that SHBG has metabolic actions of its own. SHBG has been identified to be part of a signal transduction pathway that mediates androgen and estrogen signaling at the cell membrane.^{34,35} Future research may reveal actions of SHBG that link it to CV risk factors directly. Future data from SWAN will include a prospective documentation of changes in hormones over time and how this relates to changes in risk factors. This will help determine the relative importance of these hormone factors with respect to changes in CV risk with the menopausal transition.

One limitation of the aforementioned studies as well as this one is the failure to take into account the total effect of the combined adrenal androgens, including DHEAS, androstenediol, and androstenedione. These hormones and their peripheral conversion products likely contribute to the total circulating bioactive androgen level and act with testosterone to further reduce SHBG levels. An FAI that incorporates these other values might prove to be most useful in linking reproductive hormones to CV risk.

There are a number of general limitations to this analysis. First is the cross-sectional nature of the data. Longitudinal CV risk factor data over a 5-year period will soon be available from SWAN. The prospective data will allow observations of how the hormone–risk factor associations change as women progress through the transition and reach their final menstrual period. The present data are limited to the period of time when these women were all still cycling. Because of this, it was necessary to choose a consistent time during the menstrual cycle when blood would be drawn. The interval from days 2 to 5 was chosen, but this may not be optimum for all hormones that were evaluated. Finally, categorization of premenopausal versus perimenopausal status is challenging. It is likely that not all women were correctly categorized with regard to menopausal stage.

In conclusion, low levels of SHBG and high levels of free testosterone are strongly associated with CV risk factors during the perimenopause, and this is consistent across racial groups. The evaluation of CV risk in women at midlife should shift away from solely focusing on declining endogenous estrogen levels and should include markers of androgens, such as SHBG, and measures of free androgen activity.

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