

Androgens Are Associated with Hemostatic and Inflammatory Factors among Women at the Mid-Life

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Goal: The goal of this study was to relate annually measured endogenous androgens to hemostatic and inflammation markers in women longitudinally.

Methods: A total of 3302 participants from the Study of Women's Health Across the Nation, aged 42–52 yr at baseline and self-identified as African-American (28%), Caucasian (47%), Chinese (8%), Hispanic (8%), or Japanese (9%) were evaluated for testosterone (T), dehydroepiandrosterone sulfate, and SHBG at four time points in 5 yr. Cardiovascular disease markers were fibrinogen, activated factor VII-c, C-reactive protein (hsC-RP), and the fibrolytic factors, plasminogen activator inhibitor type 1 (PAI-1), and tissue plasminogen activator [t(PA)].

Results: T and free androgen index (FAI) were associated highly

positively with PAI-1 and t(PA), and FAI was associated highly and positively with hsC-RP. Lower SHBG levels, associated with greater bioavailable T, were associated significantly with higher levels of PAI-1, t(PA), hsC-RP, and factor VII-c. SHBG was lower in Chinese and Japanese women markedly, resulting in FAI values that, on average, were higher among Chinese and Japanese women compared with African-American, Caucasian, and Hispanic women.

Implications: There were strong, positive associations of androgens with fibrolytic and inflammation markers, even after considering age, body size, smoking, and race/ethnicity. It is important to study androgens, their precursors, and their carrier protein as part of the risk profile for heart disease in mid-aged women. (*J Clin Endocrinol Metab* 90: 6064–6071, 2005)

BECAUSE WOMEN ARE relatively protected from coronary heart disease mortality in the mid-life, compared with age-matched men (1), studies of hormone mechanisms to understand this protection have tended to focus on investigating the role of estrogens and have neglected the potential role for androgens. There is a paucity of studies considering the role of androgens and coronary heart disease risk factors in women. Furthermore, the limited numbers of studies that have related testosterone (T) to heart disease or its intermediate markers in women are conflicting. For example, serum total T levels were lower in women with coronary artery disease than controls (2), not higher. Studies of intimal medial thickness have reported that higher total T values were protective for atherosclerosis in postmenopausal women (3) or a mix of pre- and postmenopausal women (4). Yet, increased free T and decreased SHBG have been associated with increased insulin concentrations and insulin resistance in a community study of Mexican-American and non-Hispanic women with a mean age of 54 yr (5). We recently reported that high serum T concentrations and free androgen index (FAI) as well as low SHBG levels were re-

lated significantly to two markers of the fibrolytic system in a cross-sectional study (6).

Some studies have suggested mechanisms by which androgens may influence hemostatic factors and, thereby, cardiovascular disease. Androgen receptors have been identified on vascular smooth muscle cells and endothelial cells (7), and other studies report a role for androgens on DNA synthesis in vascular cells (8). Recent evidence indicates that androgen treatment increases the expression of atherosclerosis genes in macrophages from male donors compared with female donors in five areas including lipoprotein processing, cell surface adhesion, extracellular processing, transport proteins, and coagulation and fibrinolysis (9). Select studies of androgens and androgen replacement suggest a capacity to modulate inflammatory cytokines produced by macrophages (10–12).

This report addresses the association of the proandrogen dehydroepiandrosterone sulfate (DHEAS), T, and the FAI (total T indexed to concentrations of the carrier protein SHBG) in relation to selected hemostatic and inflammation factors assessed at four time points in 5 yr among women at the mid-life. The selected factors include the acute phase protein, C-reactive protein (hsC-RP); the fibrolytic factors, plasminogen activator inhibitor type 1 (PAI-1) and tissue plasminogen activator [t(PA)]; and the coagulation factors, fibrinogen and factor VII-c.

Subjects and Methods

Population

The Study of Women's Health Across the Nation is a prospective, multiethnic, multidisciplinary study of the menopausal transition being

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Abbreviations: BMI, Body mass index; CV%, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index; hsC-RP, C-reactive protein; HT, hormone therapy; PAI-1, plasminogen activator inhibitor type 1; T, testosterone; t(PA), tissue plasminogen activator.

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conducted in Boston; Chicago; the Detroit area; Los Angeles; Hudson County, NJ; Pittsburgh; and Oakland, CA. Women were recruited in a two-stage process beginning with a 15-min cross-sectional survey of 16,065 women aged 40–55 yr who lived in the geographical area defined by the clinic sites. This served as the sampling frame to identify a longitudinal cohort of 3302 menstruating women, aged 42–52 yr. Women in the longitudinal cohort did not use exogenous hormone preparations that could affect ovarian function in the 3 months before enrollment, had at least one menstrual period in the 3 months before enrollment, and self-identified with the designated race/ethnic group of the site. These criteria precluded enrollment, in the longitudinal cohort, of women who were postmenopausal (natural or surgical), using oral contraceptives, or using hormone replacement.

Designated ethnic groups (African-American women at Boston, Chicago, the Detroit area, and Pittsburgh as well as Japanese, Chinese, and Hispanic women at Los Angeles, Oakland, and Hudson County, NJ, respectively) were enrolled along with Caucasian women at each site. Information about the eligibility criteria, sampling frames, and participant characteristics have been published (13). Data were collected via protocols reviewed and endorsed by an appropriate Institutional Review Board at each site. This report is based on the data from four of the six annual examinations having measures of the hemostatic and inflammation factors (baseline and follow-up first, third, and fifth examinations).

Assays

At baseline, blood was drawn during d 2–5 of the follicular phase of the menstrual cycle after an 8-h fast (96% of baseline participants). In subsequent follow-up examinations, blood draws became increasingly less likely to occur in the d 2–5 follicular phase window because the timing of menstrual bleeding became increasingly unpredictable in

women transitioning to menopause (seen in Table 1). Blood was maintained for up to an hour at 4°C until separated and then spun. Serum was frozen at –80°C and sent on dry ice to either the Clinical Laboratory Improvement Amendments-certified CLASS laboratory at the University of Michigan (T, SHBG, DHEAS) or to the Medical Research Laboratories (fibrinogen, hsC-RP, t(PA), PAI-1, and factor VII-c) for analysis. Throughout the study, the Medical Research Laboratories participated in the certification program of the National Heart Lung and Blood Institute (14).

Tissue-type plasminogen activator antigen [t(PA)-ag] was measured in plasma using a double antibody in an ELISA (IMUBIND tPA ELISA; American Diagnostica, Greenwich, CT). The assay uses human single-chain t(PA) as a standard calibrated against an international standard (NIBSAC, Hertfordshire, UK). Monthly interassay coefficients of variation (CV%) were 4.7–8.7% and 3.8–7.8% at mean concentrations of 5.6 and 11 ng/dl, respectively.

Plasma PAI-1 was measured with a sandwich procedure using a solid phased monoclonal antibody and an enzyme-labeled goat second antiserum (IMUBIND plasma PAI-1 ELISA; American Diagnostica). Monthly interassay CV% were 5–9% and 4–9% at mean concentrations of 7 and 22.5 ng/dl, respectively.

Fibrinogen was measured in frozen citrated plasma on an MLA ELECTRA 1400C (Medical Laboratory Automation Inc., Mt. Vernon, NY) using a clot-based turbidometric detection system. Monthly interassay CV% were 2.3–3.5% and 2.6–3.6% at mean concentrations of 250 and 140 mg/dl, respectively.

Factor VII-c activity was measured in frozen citrated plasma on the MLA ELECTRA 1400C (Medical Laboratory Automation Inc.) using a turbidometric detection system and using factor VII-deficient plasma (George King Bio-Medical, Overland Park, KS) in preparation of the

TABLE 1. Reproductive hormones [means (\pm SD) or median, interquartile range (IQR)] at the four visits over a 5-yr period

	Baseline visit	Visit 1	Visit 3	Visit 5
Time since base visit (yr)	0	1.03 \pm 0.17	3.06 \pm 0.19	5.05 \pm 0.20
Age (yr)	46.4 \pm 2.7	47.5 \pm 2.7	49.5 \pm 2.7	51.6 \pm 3.4
Body size measures				
BMI (kg/m ²)	27.4 \pm 6.5	27.3 \pm 6.5	27.7 \pm 6.6	28.0 \pm 6.6
Waist (cm)	86.3 \pm 15.2	86.6 \pm 15.2	87.5 \pm 15.4	88.7 \pm 15.6
Hip (cm)	106.9 \pm 14.1	107.0 \pm 14.1	107.1 \pm 14.1	107.6 \pm 14.1
Waist:hip ratio	0.80 \pm 0.07	0.81 \pm 0.07	0.81 \pm 0.07	0.82 \pm 0.07
Blood draw in early follicular phase [n (%)]				
Days 2–5 ^a	2573 (78.3)	1753 (63.8)	1113 (47.8)	590 (27.1)
Not in d 2–5 or unknown	709 (21.6)	780 (28.4)	819 (35.2)	1112 (51)
No sample	4 (0.1)	12 (0.4)	5 (0.2)	38 (1.7)
Androgens (median, IQR)				
SHBG (nM)				
In 2–5	41.6, 28–58	42.8, 28–60	39.9, 27–54	42.9, 28–61
Not in 2–5 ^a	38.7, 26–55	39.7, 26–57	36.3, 24–52	39.2, 27–57
Using HT		56.8, 37–87	56.5, 34–80	59.8, 39–91
Testosterone [ng/dl (nmol/liter)]				
In 2–5	40.6, 29–55 (1.41, 1.0–1.9)	36.4, 27–49 (1.26, 0.9–1.7)	31.9, 22–45 (1.11, 0.8–1.6)	33.5, 25–44 (1.16, 0.9–1.5)
Not in 2–5	45.4, 32–60 (1.57, 1.1–2.1)	41.6, 29–56 (1.44, 1.0–1.9)	38.2, 27–52 (1.32, 0.9–1.8)	37.6, 26–49 (1.30, 0.9–1.7)
Using HT	35.1, 23–51	31.0, 20–44 (1.22, 0.8–1.8)	31.0, 21–42 (1.11, 0.7–1.5)	31.0, 21–42 (1.08, 0.7–1.5)
FAI				
In 2–5	3.4, 2.1–5.8	3.0, 1.8–5.2	2.9, 1.6–4.8	2.7, 1.7–4.6
Not in 2–5	4.3, 2.5–6.6	3.7, 2.1–6.2	3.7, 2.2–5.9	3.3, 1.9–5.4
Using HT		2.1, 1.2–3.9	2.0, 1.1–3.6	1.8, 1.0–3.3
DHEAS [μ g/dl (μ mol/liter)]				
In 2–5	114.2, 75–170 (3.1, 2.0–4.6)	118, 77–173 (3.2, 2.1–4.7)	124.2, 82–179 (3.4, 2.2–4.9)	121.6, 83–174 (3.3, 2.2–4.7)
Not in 2–5	111.8, 73–165 (3.0, 1.9–4.5)	110, 72–170 (3.0, 1.9–4.6)	120.4, 74–176 (3.3, 2.0–4.8)	110.7, 69–164 (3.0, 1.9–4.4)
Using HT		101.3, 65–155 (3.1, 1.8–4.2)	111.4, 71–166 (3.0, 1.9–4.5)	110.1, 67–168 (3.0, 1.8–4.6)

^a Days 2–5 refers to the frequency in which phlebotomy occurred in the d 2–5 window of the early follicular phase of the menstrual cycle.

standard curve. Monthly interassay CV% were approximately 7.8, 5, and 4% for mean activities of 8, 45, and 99%, respectively.

hsC-RP was quantified using an ultrasensitive rate immunonephelometric method (hsC-RP on BN 100; Dade-Behring, Marburg, Germany). The method is based on monitoring light scattering during agglutination of hsC-RP to polystyrene particles coated with monoclonal antibodies to hsC-RP. The sensitivity of the assay (lowest detectable concentration) is 0.03 mg/dl. The CV% at hsC-RP concentrations of 0.05 and 2.2 mg/dl were 10–12% and 5–7%, respectively.

The *de novo* two-site chemiluminescent assays for serum SHBG and DHEAS concentrations involved competitive binding of 2-dimethylaminoethanol-labeled SHBG or DHEAS to a commercially available rabbit anti-SHBG or anti-DHEAS antibody and a solid phase of goat antirabbit IgG conjugated to paramagnetic particles. Inter- and intra-assay coefficients of variation for SHBG were 9.9 and 6.1%, respectively, and the lower limit of detection was 2 nM. Inter- and intra-assay coefficients of variation for DHEAS were 11.3 and 7.6%, respectively.

T concentrations were evaluated with the ACS:180 total T assay modified to increase precision in the low ranges. Serum T concentrations were determined by competitive binding of a 2-dimethylaminoethanol-labeled T derivative to a rabbit polyclonal anti-T antibody premixed with monoclonal antirabbit IgG antibody immobilized on the solid phase paramagnetic particles. Inter- and intra-assay coefficients of variation were 10.5 and 8.5%, respectively, and the lower limit of detection was 2 ng/dl. Total T was indexed to SHBG to calculate the FAI [$100 \times T$ (nanograms per deciliter)/ $28.84 \times$ SHBG (nanomolar)].

Measures

Height (centimeters) and weight (kilograms) was measured with stadiometers and calibrated scales, and used to calculate body mass index [BMI: weight (kilograms)/height (meters squared)]. Waist-to-hip ratio was calculated using hip and waist circumference measures (centimeters). Diabetes was defined as a blood glucose concentration greater than 125 mg/dl and/or use of medication for diabetes. Self-administered questionnaires were used to assess current smoking status. Physical activity (a summary score of active living, home, recreational physical activity, plus work activity, if relevant) was estimated by modifying a validated instrument and reducing the number of characterized activities from five to three (15, 16). Menopausal status was based on self-report of bleeding in the previous 3 months with decreased predictability in the time between menses (early perimenopausal) or bleeding with no decreased predictability in the same time period (premenopausal), no menses for 3–11 months (late perimenopausal), or no menses for 12 or more months (postmenopausal).

Data management and statistical analysis

Variables for fasting status, time-of-day of blood draw, and day of blood draw (d 2–5 of the early follicular phase) were evaluated for potential inclusion in statistical models, because t(PA) and PAI-1 had significant diurnal variation and values were higher in women whose blood was drawn outside of d 2–5 of the menstrual cycle. A variable for site and ethnicity was included in all models to account for sampling design. Continuous variables, other than age, were transformed (log or square root) to satisfy model assumptions including normally distributed residuals.

Data from women being treated with anticoagulants ($n < 10$) were excluded from analyses for the particular examination in which women reported using anticoagulants. Data from women who reported using aspirin were retained in data analyses because the reason for aspirin use could not be discerned explicitly and there were no differences in the cardiovascular disease markers in those women that reported aspirin use *vs.* those who reported no use. When hormone therapy (HT) use was reported at postbaseline examinations, those data were censored. Women with surgical menopause were censored at the time of the report of hysterectomy, because the ovarian theca cells produce androgens.

To describe hemostatic factor concentrations over the 5-yr period in association with androgens, we used longitudinal mixed models, which can be specified to account for the autocorrelation in repeated measures. Models used values of the hemostatic or inflammation markers (as time-varying measures) as the dependent variable, whereas measures of the androgens were the primary independent variables (as time-varying

measures). Age (as a time-varying covariate) was included in the models as a covariate or with the individual androgen in an interaction term to identify whether the association of the androgen and the hemostatic factor changed with age. Waist circumference, BMI, and being in or out of the blood draw window were evaluated in models as time-varying covariates. Variables for self-reported race/ethnicity, site, and baseline smoking behavior were entered into models as time-invariant covariates. The relationships between androgens and the hemostatic factors are shown in bar graphs to facilitate the interpretation of the β coefficients with cutpoints established at approximately quartiles for most measures and sextiles for DHEAS and SHBG where there was a wider distribution of values.

Model fit was assessed using residual analyses and the Akaike criteria. Log transformations were untransformed for presentation in graphs. A value of $P < 0.01$ was considered statistically significant. All longitudinal models were fit with a random intercept and fixed independent effects. The assumed longitudinal correlation structures for random and fixed effects were unstructured and variance components, respectively.

Results

Tables 1 and 2 show the unadjusted cross-sectional mean values of the hemostatic factors and androgens, including FAI, at each of the four examinations and according to the presence or absence of HT. As seen in Table 3, at baseline, 54% of women were classified as premenopausal and the remaining 46% were in early perimenopause. By the fifth follow-up examination, only 5% of women remained classified as premenopausal. At baseline, women did not use HT; however, by the fifth follow-up examination, 20% of women, cumulatively, had used HT.

After excluding HT users, there were significantly higher concentrations of the proandrogen DHEAS among Chinese women compared with Japanese, African-American, Caucasian, and Hispanic women at each visit, as shown in Fig. 1. Furthermore, as seen in Fig. 1, SHBG was markedly lower in Chinese and Japanese women. When SHBG was placed in the ratio with total T (FAI), this indicator of bioavailable T was remarkably higher, on average, among Chinese and Japanese women. Because it is recognized widely that SHBG concentrations are lower as body size increases, the data shown in Fig. 1 were adjusted for BMI and smoking behavior. These relationships provided the rationale for adjusting for ethnicity and BMI, as well as censoring for eventual HT use and surgical menopause, with the impact of HT shown in Table 1. The following associations between the androgens and the hemostatic factors were adjusted for age, BMI, race/ethnicity, site, and smoking status.

Fibrolytic markers

Higher levels of T and FAI were associated with higher levels of the fibrolytic markers PAI-1 and t(PA), shown in Table 4. As seen in Fig. 2, higher androgen concentrations, including FAI ($\beta_{\log} = 0.26$, $P < 0.0001$) and DHEAS ($\beta_{\log} = 0.011$, $P < 0.0006$) were associated significantly with higher PAI-1 concentrations. SHBG concentrations were associated negatively with PAI-1.

As seen in Fig. 3, androgens, including T, FAI, and DHEAS ($\beta_{\text{sqr}} = 0.004$, $P < 0.006$) were associated highly and positively with t(PA). SHBG concentrations were associated significantly and negatively with t(PA).

TABLE 2. Hemostatic factors (median, interquartile range) at the four visits over a 5-yr period

	Baseline visit	Visit 1	Visit 2	Visit 3
hsC-RP [mg/liter (nmol/liter)]				
Using HT	N/A	2.20, 0.80–5.10 (102.3, 37–237)	2.40, 1.00–5.70 (111.6, 46–265)	2.50, 1.00–6.40 (116.2, 46–298)
Not using HT	1.60, 0.60–4.70 (74.4, 28–219)	1.40, 0.60–4.20 (65.1, 28–195)	1.60, 0.60–4.70 (74.4, 28–219)	1.70, 0.60–4.65 (79.0, 28–216)
Fibrinogen [mg/dl (μ mol/liter)]				
Using HT	N/A	267, 238–308 (7.85, 7.0–9.05)	267, 238–300 (7.85, 7.0–8.82)	267, 238–300 (7.85, 7.0–8.82)
Not using HT	282, 246–336 (8.29, 7.23–9.88)	273, 238–316 (8.03, 6.9–9.29)	273, 242–316 (8.03, 7.1–9.29)	273, 238–308 (8.03, 7.0–9.06)
PAI-1 [ng/ml (pmol/liter)]				
Using HT	N/A	13.9, 8.9–27.0 (278, 179–541)	16.4, 8.5–29.8 (328, 170–596)	10.6, 6.1–18.3 (212, 122–366)
Not using HT	20.8, 12.2–34.3 (416, 244–686)	18.5, 10.3–31.8 (370, 206–636)	20.8, 10.9–39.1 (416, 218–782)	15.4, 8.8–27.7 (308, 175–554)
t(PA) [ng/ml (pmol/liter)]				
Using HT	N/A	7.45, 5.4–9.7 (106.5, 77–139)	6.70, 4.7–9.0 (95.8, 67–129)	6.10, 4.5–7.9 (87.2, 64–113)
Not using HT	7.30, 5.3–9.7 (104.4, 76–139)	7.85, 5.6–10.5 (112.3, 80–150)	7.40, 5.3–9.8 (105.8, 76–140)	7.20, 5.4–9.3 (103.0, 77–133)
Factor VIIc (%)				
Using HT	N/A	118, 96–140	129, 110–149	124, 103–146
Not using HT	115, 98–133	111, 96–127	117, 103–136	118, 101–135

N/A, Not applicable.

hsC-RP

Total T ($\beta_{\log} = -0.091$, $P < 0.0001$) and SHBG ($\beta_{\text{sqr}} = -0.060$, $P < 0.0001$) were associated significantly and negatively with hsC-RP, and, as a result, FAI was associated with higher hsC-RP levels at any given time ($\beta_{\log} = 0.042$, $P < 0.007$), as shown in Fig. 4. There was no significant association of DHEAS with hsC-RP concentrations.

Coagulation markers

Androgens were related less strikingly to the coagulation markers. Factor VII-c was associated negatively with SHBG concentrations ($\beta_{\text{sqr}} = -0.009$, $P < 0.0001$). This led to a significant and positive association of factor VII-c with FAI ($\beta_{\log} = 0.012$, $P < 0.002$). There was no significant association of total T with factor VII-c.

DHEAS was the only androgen positively associated with fibrinogen ($\beta_{\log} = 0.003$, $P < 0.0006$). T, SHBG, and FAI were

not associated significantly with fibrinogen, as reported in Table 4.

Discussion

We identified that higher FAI and lower SHBG were associated with significantly less favorable fibrolytic [PAI-1 and t(PA)] levels in these mid-aged women. Likewise, higher androgens were associated with higher and less favorable hsC-RP levels, although the proandrogen DHEAS was not.

Other studies have shown mixed findings in relating androgens to heart disease. Phillips *et al.* (17, 18) reported that free T and cholesterol were predictors of atherosclerosis, defined by angiography, in a small sample of postmenopausal women, many of whom had experienced a previous myocardial infarction. However, other research among postmenopausal women reported no strong associations between blood concentrations of lipids and free or total T (19).

TABLE 3. Number and frequency of women with hemostatic measures seen at each visit according to menopause status, race/ethnicity, and diabetes

	Baseline	Visit 1	Visit 3	Visit 5
Total no.	3286	2747	2329	2171
Menopause status				
Pre-menopausal	1758 (54)	693 (25)	262 (11)	100 (5)
Early perimenopausal	1496 (46)	1656 (60)	1217 (52)	813 (37)
Late perimenopausal	0	117 (4)	201 (9)	235 (11)
Postmenopausal	0	56 (2)	227 (10)	540 (25)
Surgical menopause	0	11 (<1)	28 (1)	50 (2)
HT use	0	202 (7)	393 (17)	441 (20)
Race/ethnicity				
Caucasian	1545 (47)	1356 (49)	1147 (49)	1029 (47)
African-American	927 (28)	697 (25)	576 (25)	601 (28)
Chinese	250 (8)	239 (9)	224 (10)	205 (9)
Japanese	285 (9)	261 (10)	245 (11)	237 (11)
Hispanic	279 (9)	194 (7)	137 (6)	108 (5)
Diabetes	239 (7)	173 (6)	156 (7)	190 (9)

Frequency is shown as percentage in parentheses.

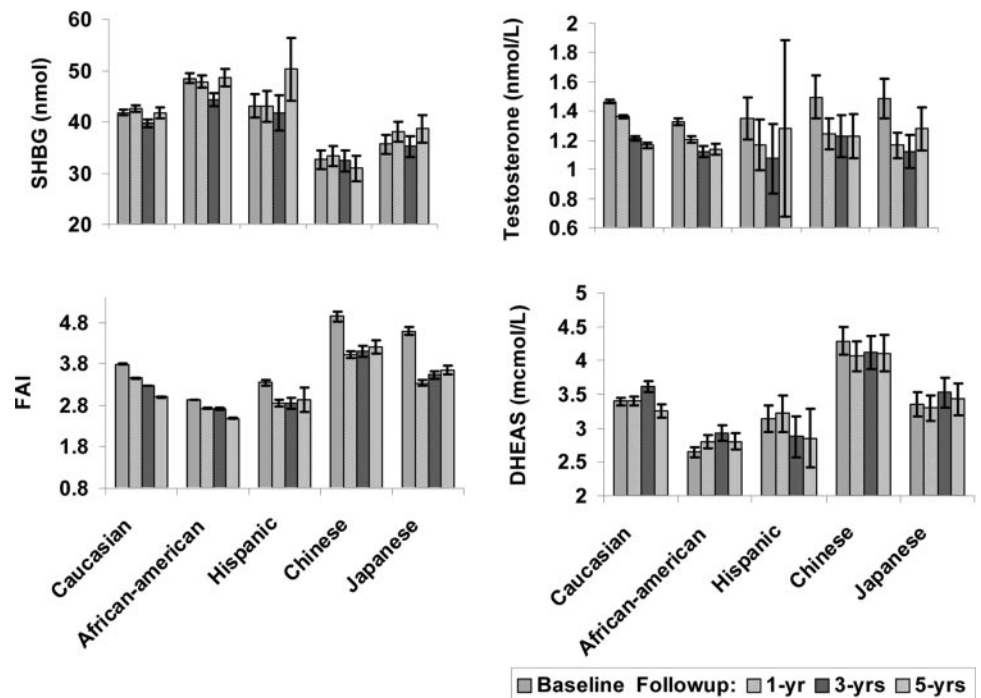


FIG. 1. Androgen levels (least square mean \pm SE) by ethnic group at each of the four visits, adjusted for age, body size, ethnicity, site, and baseline smoking status; HT users excluded.

In the Rancho Bernardo study, there was no association between total or bioavailable T and cardiovascular disease deaths (20). However, the same study has contributed to the controversial history of the proandrogen, DHEAS, in part, based on a 1986 report that men, but not women, with a lower DHEAS level were more likely to experience a premature death associated with cardiovascular disease (21, 22). Two reports indicate an association of T and hypertension in pre- and postmenopausal women (17, 23).

We identified strong associations of androgens and PAI-1, t(PA), and hsC-RP, based on data from a large group of women measured over time. The carrier protein, SHBG, was the most strongly associated element. It is well-recognized that androgens circulate in free and bound states, in which the bound hormone is inhibited from diffusing across cell membranes to initiate hormone actions. Given that only 1–2% of T is estimated to be available biologically (24), we adopted the common practice of using FAI, the ratio of total T and SHBG, to approximate the free moiety.

SHBG could have a number of important roles with respect to cardiovascular disease. SHBG can regulate bioavailable androgen by binding with high affinity to T and dihydrotestosterone, which could result in more available estrogen. SHBG can regulate intracellular physiology by acting like a hormone (5), a role supported by studies indicating the presence of SHBG receptors that respond by increasing cAMP (25). Finally, SHBG and hemostatic factors are synthesized and secreted by hepatocytes, with evidence of shared homology between SHBG and the blood clotting cascade (26) and the fibrolytic system, particularly PAI-1 (27, 28). SHBG may behave like serine protease inhibitors as a substrate for elastase and cleaved by activated neutrophils, conditions seen in obesity and insulin resistance (29).

The competing elements of race/ethnicity and body size made it important to adjust for these covariates. As expected, SHBG concentrations declined with increasing body size (30). However, the SHBG concentrations were also lower among the Chinese and Japanese women who were smaller,

TABLE 4. Modeled associations between androgens and hemostatic factors, from longitudinal models and adjusted for covariates^a

	Hormone concentrations							
	\log_{10} Testosterone β (P value)		\log_{10} FAI β (P value)		$\sqrt{\text{SHBG}}$ β (P value)		$\sqrt{\text{DHEAS}}$ β (P value)	
	Main effect	Interact with age	Main effect	Interact with age	Main effect	Interact with age	Main effect	Interact with age
\log_{10} PAI-1	0.092 ^b	NS	0.26 ^b	0.014 ^b	-0.128 ^b	-0.008 ^b	0.011 ^c	NS
\log_{10} t(PA)	0.082 ^b	NS	0.091 ^b	NS	-0.032 ^b	NS	0.004 ^d	NS
\log_{10} Factor VII-c	NS		0.012 ^e	NS	-0.009 ^b	NS	NS	
\log_{10} Fibrinogen	NS		NS		NS		0.003 ^c	NS
\log_{10} C-RP	-0.091 ^b	NS	0.042 ^c	NS	-0.060 ^b	NS	NS	

^a Final reduced models after evaluation with age as the time marker, a quadratic term with age, interaction terms with age \times hormone, as well as covariates including BMI, race/ethnicity, site, day of blood draw, and smoking. NS, Not significant.

^b $P < 0.0001$.

^c $P < 0.001$.

^d $P < 0.01$.

^e $P < 0.005$.

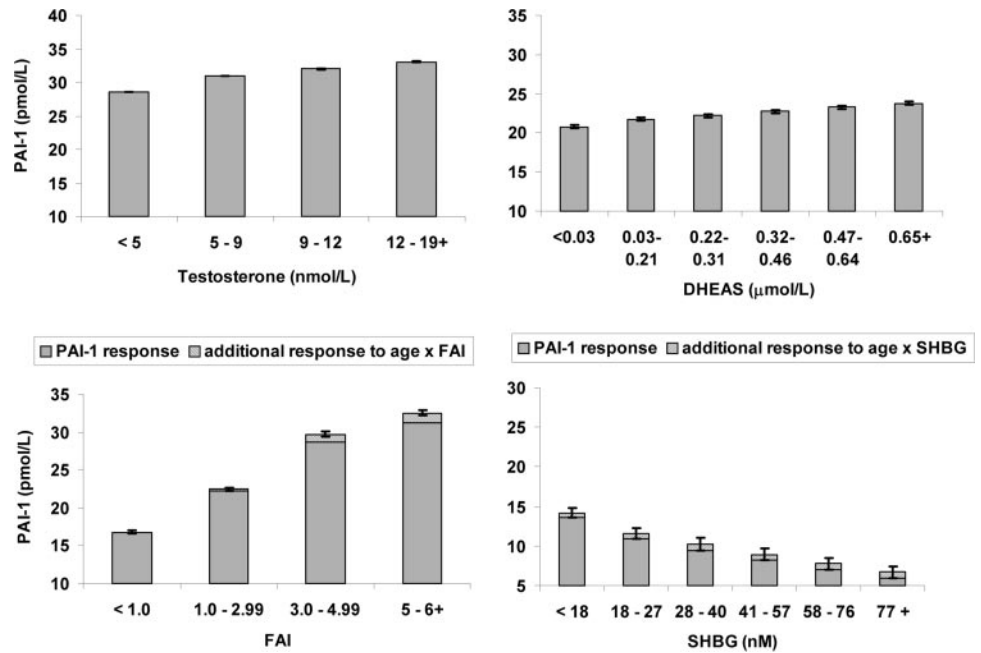


FIG. 2. Modeled longitudinal associations between PAI-1 and hormones, adjusted for covariates, as reported in Table 3. Age × hormone response shown for age = 50 yr.

on average, than Caucasian, African-American, and Hispanic women, even after adjusting for differences in BMI and smoking behavior. This suggests that unappreciated endocrinological, lifestyle, or genetic differences need to be considered before considering causal modeling for the role of androgens, the carrier proteins, and hemostatic factors.

The relative absence of studies of androgens and cardiovascular disease among women during the menopause transition means that statistical models to describe associations tended to be more grounded in phenomenology rather than true hypothesis testing. In evaluating statistical models, the associations tended to be main effects, akin to a correlation, with very few instances of significant interaction terms that indicated that the hemostatic factor concentrations increased

or decreased significantly with the annually measured hormone concentrations. The only significant age interaction, suggesting a change with time (with age acting as the surrogate for time), was the association of SHBG and PAI-1. These interactions indicated that there was a more pronounced association of FAI and PAI-1 with age, but a less pronounced association of SHBG and PAI-1 with increasing age or greater observed time. Potentially, the reason that other factors were not associated with a change with time was that there was too limited a time to be able to observe a change, if it existed.

DHEAS, a proandrogen requiring conversion to T to express its androgenic characteristics (31), was related to the fibrolytic factors as well as fibrinogen. DHEAS had been

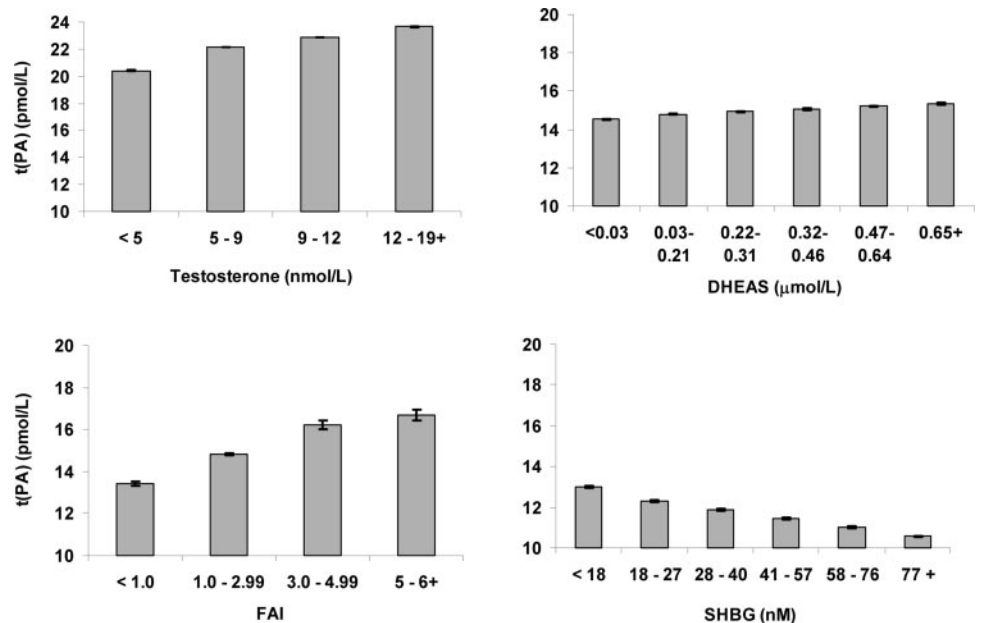


FIG. 3. Modeled longitudinal associations between t(PA) and hormones, adjusted for covariates, as reported in Table 3.

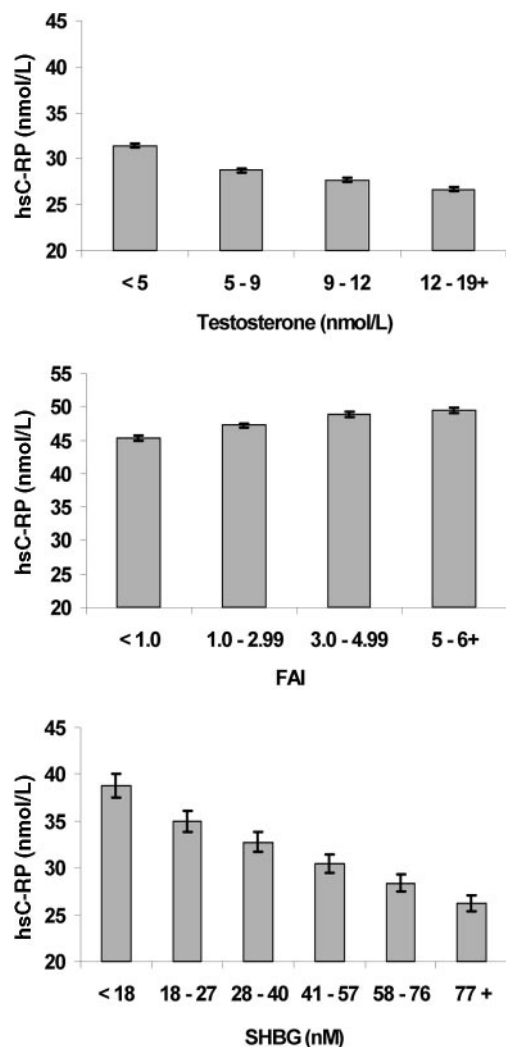


FIG. 4. Modeled longitudinal associations between hsC-RP and hormones, adjusted for covariates, as reported in Table 3.

reported previously as being related to cardiovascular disease mortality in men, but not women (22). Although total T concentrations reflect approximately equal contributions from the ovary and the adrenals, it is estimated that more than 90% of DHEAS is a secretory product of the adrenal zona reticularis. Paradoxically, it had been reported that circulating concentrations do not change significantly with the menstrual cycle or the menstrual transition, while simultaneously being modulated by estrogen concentrations (31), which certainly change in circulating levels during both the menstrual cycle and the menopause. This suggests the need for examining other members of the androgen pathway in relation to estrogens to better understand the dynamics of the relationships with the hemostatic factors.

This study includes several strengths and limitations. Although androgens were related to the fibrolytic markers and hsC-RP, the study does not include a direct measure of bioavailable T. Furthermore, the measures of endogenous serum hormones are a limited representation of the bioavailable hormone fraction that crosses cellular membranes and binds to nuclear steroid receptors (32). The serum immunoreactive

assay absolute value may be higher than assays now available that involve expensive and time-consuming extraction-based methodology; however, these methodologies would not be feasible in large population-based, longitudinal studies. The annual specimen collection was timed to a day-of-cycle window, when possible, as well as time of day, but findings must be interpreted with caution given the cyclic and pulsatile nature of circulating endogenous hormones in transitioning women. This study includes a substantially sized population of women being followed across time in the age range of the menopausal transition. Members of the group were either pre- or early-perimenopausal (based on menstrual bleeding definitions) and without use of HT at the study onset, helping to establish the temporality of transitional events. Whether these hemostatic factors, measured at the time of the transition, will have a substantial impact on hard endpoints remains to be described. Currently, the age and size of the population are insufficient to generate sufficient hard cardiovascular endpoints with adequate power to detect important contributions.

In summary, the role of hormones, especially androgens, on the hemostatic risk factors for heart disease have been evaluated inadequately. This study documented that T and FAI were associated highly and negatively with the fibrolytic factors as well as with hsC-RP. However, the impact that race/ethnicity and body size have on the association between SHBG and its binding capacity with total T suggest care in extrapolating results. The associations of higher T and higher hemostatic markers, even following adjustment for age, body size, smoking, and ethnicity, are intriguing and highlight the importance of studying androgens and their precursors as part of the risk profile for the development of heart disease in mid-aged women.

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