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Endogenous Postmenopausal Hormones and Carotid Atherosclerosis: A Case-Control Study of the Atherosclerosis Risk in Communities Cohort

Sherita Hill Golden,[1] Ann Maguire,[2] Jingzhong Ding,[3] J. R. Crouse,[4] Jane A. Cauley,[5] Howard Zacur,[6] and Moyses Szklo[3]

Studies examining the relation between endogenous postmenopausal hormone levels and cardiovascular disease have yielded conflicting results. After excluding women with a history of hormone replacement therapy (HRT) use, the authors conducted a US case-control study in 1987–1992 comparing endogenous postmenopausal hormone levels in women with and without significant carotid atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) cohort. Atherosclerosis was assessed by using B-mode ultrasound to measure carotid artery intimal-medial thickness (IMT). Cases (n = 182) were postmenopausal women with average IMT measurements ≥ the 95th percentile. Controls (n = 182) were frequency matched to cases on age and ARIC center and had IMT measurements < the 75th percentile. After adjustment for cardiovascular risk factors, no association was found between the odds of atherosclerosis and increasing quartiles of estrone, dehydroepiandrosterone sulfate, or androstenedione. Compared with participants in the lowest quartile of sex hormone-binding globulin (SHBG), those in the highest quartile had a significantly lower odds of atherosclerosis (odds ratio = 0.48, 95% confidence interval: 0.24, 0.97). Similarly, participants in the highest quartile of total testosterone had a lower odds of atherosclerosis (odds ratio = 0.38, 95% confidence interval: 0.20, 0.74). The authors found higher total testosterone and SHBG to be inversely related to carotid atherosclerosis, suggesting their potential importance in reducing atherosclerotic risk in postmenopausal women not using HRT. *Am J Epidemiol* 2002;155:437–45.

androgens; cardiovascular diseases; carotid arteries; estrogen replacement therapy; estrogens; postmenopause; sex hormone-binding globulin

In observational studies, postmenopausal women using estrogen have been found to have a reduced risk of major clinical coronary disease and reduced cardiovascular disease mortality (1). However, a large, randomized clinical trial of estrogen replacement therapy for secondary prevention of coronary heart disease in postmenopausal women failed to demonstrate a reduced rate of coronary heart disease events (2). Finally, two studies examining the relation of estrogen replacement therapy to carotid atherosclerosis have yielded conflicting results (3, 4).

Several studies have shown beneficial cardiovascular effects of exogenous estrogen, including lower body weight (4–6), increased high density lipoprotein (HDL) cholesterol and lower low density lipoprotein cholesterol (4, 7–9) levels, lower fasting insulin and glucose levels (4, 7), improved brachial artery blood flow (10), and regression of atherosclerotic plaques (11). Estrogen replacement therapy results in a higher sex hormone-binding globulin (SHBG) level (12), which is associated with less insulin resistance and a more favorable cardiovascular risk factor profile (13–17), even in postmenopausal women not using hormone replacement therapy (13, 16, 17).

Prior to menopause, women have a much lower risk of cardiovascular disease compared with men of the same age (18); however, menopause initiates a phase of increased risk (18). At the time of menopause compared with the premenopausal state, a woman's endogenous hormonal milieu changes; there is a relative estrogen deficiency and a relative increase in testosterone levels. A weaker form of estrogen, estrone, continues to be synthesized as a result of peripheral conversion from adrenal androstenedione in the fat, liver, and kidney (19). Given what is known about the relation

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Abbreviations: ADIOL, 5-androstene-3β, 17β-diol; ARIC, Atherosclerosis Risk in Communities; CI, confidence interval; DHEA-S, dehydroepiandrosterone sulfate; HDL, high density lipoprotein; OR, odds ratio; SHBG, sex hormone-binding globulin.

[1] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.
[2] Department of Medicine, University of Wisconsin School of Medicine, Milwaukee, WI.
[3] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.
[4] Department of Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.
[5] Department of Medicine, University of Pittsburgh School of Public Health, Pittsburgh, PA.
[6] Department of Obstetrics and Gynecology, Johns Hopkins University School of Medicine, Baltimore, MD.
Correspondence to Dr. Sherita Hill Golden, Division of Endocrinology and Metabolism, Johns Hopkins University School of Medicine, 1830 East Monument Street, Suite 333, Baltimore, MD 21205 (e-mail: sahill@jhmi.edu).

between estrogens, androgens, and cardiovascular disease in women receiving estrogen replacement therapy, postmenopausal women with significant atherosclerosis and cardiovascular disease would be expected to have lower endogenous estrogen levels and higher endogenous androgen levels than those without significant atherosclerosis.

Studies examining the relation between endogenous postmenopausal hormone levels and cardiovascular disease have yielded conflicting results. Several studies have found no association between sex hormone levels and risk of death from cardiovascular disease (20–24). However, two studies suggest a protective effect of higher androgen levels on cardiovascular disease in postmenopausal women (25, 26). With these findings in mind, we conducted a case-control study to compare endogenous postmenopausal hormone levels in women with and without significant carotid atherosclerosis.

MATERIALS AND METHODS

Study population

The Atherosclerosis Risk in Communities (ARIC) cohort is a probability sample of 15,792 men and women aged 45–64 years from four US communities (selected Minneapolis suburbs, Minnesota; Washington County, Maryland; Forsyth County, North Carolina; and Jackson, Mississippi). Details of the sampling frames and methods and of the cohort examination procedures have been described previously (27).

Atherosclerosis cases and controls were chosen on the basis of their average carotid artery ultrasound measurements from ARIC field center visits 1 and 2, conducted 3 years apart, in 1987–1989 and 1990–1992, respectively. Postmenopausal women in the ARIC cohort who were not current or ever users of hormone replacement therapy were eligible for the present analysis. Menopause was defined on the basis of visit 1 interview data. A woman was considered postmenopausal if she had not menstruated in the last 2 years. Postmenopausal women were further classified as having undergone surgical menopause if they had had a bilateral oophorectomy. Natural menopause also described nonmenstruating women 55 years of age or older who had had a hysterectomy and had at least one intact ovary (28).

Exposure

Endogenous postmenopausal hormone status was assessed by measuring levels of estrone, androgens (androstenedione, dehydroepiandrosterone sulfate (DHEA-S), and total testosterone), and SHBG; testing was performed by Yerkes Laboratory on blood collected during visit 2, because visit 1 serum was not available (Assay Services Laboratory, Yerkes Regional Primate Research Center of Emory University, Atlanta, Georgia). Estrone was measured instead of estradiol because of concern that the assay might not be able to detect small differences in estradiol levels in postmenopausal women. Serum androstenedione, DHEA-S, and testosterone were measured by radioimmunoassay using a Diagnostics Products Corporation kit (Los Angeles, California). Serum estrone and SHBG were measured by radioimmunoassay using a Diagnostic Systems Laboratory assay kit (Webster, Texas). The intra-assay coefficient of variation for each hormone was <10 percent. The interassay coefficients of variation were 9 percent for DHEA-S and total testosterone, 13 percent for estrone, 16 percent for androstenedione, and 18.5 percent for SHBG. The lower limits of detection for estrone, androstenedione, DHEA-S, total testosterone, and SHBG were 7.5 pg/ml, 0.1 ng/ml, 2.5 μg/dl, 5 ng/dl, and 5 nmol/liter, respectively. We also calculated the total testosterone/SHBG ratio as a marker of free testosterone (29). All assays were performed in the same batch for cases and controls.

Definition of cases and controls

Carotid atherosclerosis was assessed by using B-mode ultrasound to measure carotid artery intimal-medial thickness (30). The ARIC study ultrasound methods use a scanning protocol common to all four field centers, with central reading according to a standardized protocol (30). Intimal-medial thickness was measured and was averaged over six different sites: bilaterally in the common carotid artery, the carotid bifurcation, and the internal carotid arteries. The within-sonographer intraclass correlation coefficient between scans repeated was 0.81. The correlation coefficient between blinded repeat readings of the same scan by different readers was 0.93 (31).

The average intimal-medial thickness for visits 1 and 2 was used to determine case-control status. Cases were defined as postmenopausal women with no history of hormone replacement therapy use who had an average of more than or equal to the 95th percentile for all carotid intimal-medial thickness measurements at each of six sites visualized during visits 1 and 2. Controls were postmenopausal women with no history of hormone replacement therapy use, frequency matched to cases by 5-year age groups and ARIC center. Because of the high prevalence of atherosclerosis in this age group, as shown in the International Atherosclerosis Project (32), we attempted to select controls who had minimal evidence of disease to avoid misclassification of case-control status. Therefore, one control was chosen per case from among those women whose intimal-medial thickness was less than the 75th percentile at each of six sites visualized, for each age–ARIC center stratum. Matching on ARIC center also largely resulted in race matching because most African-American participants were recruited at the Jackson, Mississippi, field center (27).

Covariates

Covariates included in the analysis were age, ARIC center, total cholesterol, HDL cholesterol, systolic blood pressure, smoking history, alcohol intake, sports index, fibrinogen, body mass index (defined as weight in kilograms divided by height in meters squared), and insulin level. Information on variables from visit 1 was used.

Blood analyses. With the participant seated, a 21-gauge butterfly needle was used to perform a minimally traumatic venipuncture the morning after participants had fasted for

12 hours. Fasting times were recorded. After standardized processing at the clinic site, samples were aliquoted into 2-ml tubes, frozen at –70°C, and shipped on dry ice to the appropriate ARIC central laboratory (30). Insulin was measured by radioimmunoassay (^{125}I Insulin 100 test kit; Cambridge Medical Diagnostics, Billerica, Massachusetts). Levels of total cholesterol and triglycerides were measured by using enzymatic methods (30), HDL cholesterol by using dextran and magnesium precipitation (30), and low density lipoprotein cholesterol by using the Friedewald formula (33). Fibrinogen levels were measured by performing coagulation tests, and the standard was a batch of universal coagulation reference plasma (34).

Physical measurements. Anthropometry was performed in the fasting state with the urinary bladder empty. Participants wore lightweight, nonconstricting underwear. Measurements were taken by teams of two certified technicians to ensure optimal placement of measuring instruments. Height (without shoes) was measured by using a wall-mounted ruler. Weight was measured with a balance scale (model #437; Detecto, Jericho, New York), which was zeroed daily (30).

After the participant had been seated for 5 minutes, blood pressure was measured in the right arm by using a random-zero sphygmomanometer and an appropriate-sized cuff. Three measurements were taken; the mean of the second and third measurements was used to characterize blood pressure at the visit (30).

Cigarette exposure. Active smoking was assessed by using a 12-item questionnaire. For the present analysis, smoking history was summarized as pack-years of smoking (30).

Alcohol intake. Current and former alcohol intake were assessed by using a dietary intake questionnaire (30).

Physical activity. Physical activity was assessed by interview using a questionnaire developed by Baecke et al. that included 16 items about usual exertion (35). Three indices ranging from 1 (low) to 5 (high) were derived for physical activity at work, during leisure time, and in sports.

Analysis

In univariate analysis, mean concentrations of each hormone were compared between cases and controls by using the Wilcoxon rank-sum two-sample test because hormone levels were not distributed normally. For the purpose of multivariate analysis, hormone levels were divided into quartiles based on the joint distribution of cases and controls. Odds ratios and 95 percent confidence intervals were calculated from multiple logistic regression models; quartile 1 was used as the reference. Multiple linear regression was also performed, in which the independent variable was case-control status, and each hormone was entered into the model as the dependent variable. The regression coefficient for the case-control indicator in each model represented the mean difference in that hormone between cases and controls. Because the main source of estrone in postmenopausal women is peripheral aromatization of androstenedione in fatty tissue (19), body mass index is in the causal pathway in the relation between estrone and carotid intimal-medial thickness. Therefore, in one set of models, body mass index was excluded in multivariate analysis; however, in another set of models, adjustment was performed for body mass index as a continuous variable to determine whether the associations between hormone levels and carotid atherosclerosis were independent of the degree of obesity. Statistical analyses were carried out by using SAS release 6.12 statistical software (36).

RESULTS

Baseline characteristics

Table 1 displays the demographic and cardiovascular risk factor characteristics for cases and controls. Mean carotid intimal-medial thickness for cases and controls was 1.06 mm (standard deviation, 0.15) and 0.71 mm (standard deviation, 0.12), respectively. Compared with controls, cases had significantly higher total cholesterol levels, systolic blood pressure, mean pack-years of cigarette smoking, and fasting glucose, fasting insulin, and fibrinogen levels and significantly lower HDL cholesterol levels. Cases also had a higher prevalence of diabetes. Body mass index and sports index were not significantly different between cases and controls. Although not statistically significant, the odds ratio for atherosclerosis was lowest among current drinkers compared with never drinkers (data not shown). Similar percentages of cases and controls had undergone surgical menopause.

Univariate analysis

Cases had significantly higher concentrations of estrone (34.0 pg/ml for cases, 29.9 pg/ml for controls) and androstenedione (0.90 ng/ml for cases, 0.81 ng/ml for controls) and significantly lower SHBG levels (70.8 nmol/liter for cases, 73.7 nmol/liter for controls) (table 2). Total testosterone and DHEA-S concentrations and total testosterone/SHBG ratio were not significantly different between cases and controls. Median hormone levels between cases and controls were nearly identical following adjustment for body mass index.

Multivariate analysis

Table 3 shows the results of two multiple logistic regression models. In the first model, after adjustment for age and ARIC center, there was a significantly greater odds of carotid atherosclerosis for those women in the highest quartile of estrone (odds ratio (OR) = 1.86, 95 percent confidence interval (CI): 1.03, 3.36). In this model, the odds of carotid atherosclerosis were significantly less than 1.0 for women in the second (OR = 0.49, 95 percent CI: 0.26, 0.86) and fourth (OR = 0.45, 95 percent CI: 0.25, 0.82) quartiles of SHBG (table 3, model 1).

In the second model, we adjusted for age and ARIC center as well as for total cholesterol, HDL cholesterol, systolic blood pressure, pack-years of smoking, alcohol intake, sports index, fibrinogen, body mass index, glucose, insulin,

TABLE 1. Selected demographic, behavioral, and physiologic characteristics of cases and controls,* Atherosclerosis Risk in Communities cohort, United States, 1987–1992

Characteristic	Controls (n = 182)	Cases (n = 182)	p value†
Age (years)‡	61.6 (4.04)	61.7 (4.01)	
African-American race (%)§	31.3	32.4	
Cigarette smoking (pack-years)	167 (298)	394 (423)	<0.001
Alcohol intake (%)			
Current	43	39	
Former	21	25	
Never	35	36	0.642
Body mass index (kg/m^2)	27.6 (5.29)	28.2 (5.41)	0.238
Total cholesterol (mg/dl)	220 (42.3)	232 (50.1)	0.008
High density lipoprotein cholesterol (mg/dl)	53.4 (15.0)	49 (16.0)	0.002
Fasting glucose (mg/dl)	117 (51.1)	136 (73.9)	0.006
Fasting insulin (pmol/liter)	97.1 (95.2)	200 (600)	0.023
Sports index	2.31 (0.70)	2.31 (0.77)	0.975
Fibrinogen (mg/dl)	314 (56.9)	334 (88.0)	0.013
Surgical menopause (%)	6.6	5.2	0.417
Diabetic (%)	7.1	13	0.01

* For continuous variables, the summary statistic is the mean (standard deviation); for categorical variables, the summary statistic is the percentage of cases and controls with the given characteristic.
† p value for Student's t test for continuous variables and χ^2 test for categorical variables.
‡ Groups were frequency matched on age.
§ Groups were matched on Atherosclerosis Risk in Communities field center/race.

TABLE 2. Unadjusted median values (ranges) of endogenous hormones for cases and controls, Atherosclerosis Risk in Communities cohort, United States, 1987–1992

Hormone	Cases	Controls	p value*
Estrone (pg/ml)	34 (114)	29.9 (65.7)	0.014
Total testosterone (ng/dl)	21.0 (440)	20.6 (460)	0.541
Androstenedione (ng/ml)	0.90 (7.7)	0.81 (4.7)	0.042
DHEA-S† (μg/dl)	69 (488)	73.6 (393)	0.609
SHBG† (nmol/liter)	70.8 (237)	73.7 (178)	0.048
Total testosterone/ SHBG ratio	0.28 (0.75)	0.29 (0.60)	0.670

* p value for Wilcoxon rank-sum two-sample test.
† DHEA-S, dehyroepiandrosterone sulfate; SHBG, sex hormone-binding globulin.

and type of menopause. There did not appear to be clear-cut association patterns for the relative odds of carotid atherosclerosis with increasing quartiles of estrone, DHEA-S, or androstenedione (table 3, model 2). However, compared with participants in the lowest quartile of total testosterone, those in the highest quartile had an odds ratio significantly lower than 1.0 (OR = 0.34, 95 percent CI: 0.16, 0.70; p = 0.004) in the fully adjusted model (table 3, model 2). A consistent graded and inverse relation was apparent with increasing quartiles of total testosterone/SHBG ratio, indicating higher bioavailability of testosterone, although the trend was not statistically significant (table 3).

Higher SHBG levels also seemed to be associated with a lower odds of carotid atherosclerosis (table 3, model 2); for those in the highest quartile, the odds ratio was 0.42 (95 percent CI: 0.20, 0.90; p = 0.04) in the fully adjusted model. Body mass index, alcohol intake, and smoking status could have changed between visits 1 and 2. Analyses performed to account for changes in these variables did not alter the association patterns.

Because body mass index, total and HDL cholesterol, and insulin levels might mediate the relation between hormones and carotid atherosclerosis, these variables were excluded from multivariate analysis. The odds ratios for the association between each hormonal variable and carotid atherosclerosis were nearly identical in models that excluded body mass index, insulin, both insulin and body mass index, and both total and HDL cholesterol (not shown in table). In subsidiary analyses stratified by body mass index ≤25 kg/m^2 and body mass index >25 kg/m^2, a similar association of higher SHBG and testosterone levels with a lower odds of carotid atherosclerosis was observed, and it reached statistical significance for those women with a body mass index of >25 kg/m^2 (not shown in table). In these stratified analyses, the association of the adrenal androgens and estrone with carotid atherosclerosis remained statistically insignificant. Stratification by body mass index ≤30 kg/m^2 or >30 kg/m^2 revealed similar patterns (not shown in table). Stratification by race (African American and White) also showed an increased odds of carotid atherosclerosis in the highest quartile of SHBG in Whites that was of borderline statistical significance. However, this pattern was not observed for African Americans, likely because of inadequate power (not shown in table).

DISCUSSION

We found that total testosterone and SHBG levels were inversely related to carotid atherosclerosis. Although not statistically significant, higher total testosterone/SHBG ratio, a marker of more elevated free testosterone, also

TABLE 3. Odds ratios and 95% confidence intervals for atherosclerosis for quartiles of estrone, DHEA-S†, androstenedione, total testosterone, total testosterone/SHBG† ratio, and SHBG in postmenopausal women not using hormone replacement therapy, Atherosclerosis Risk in Communities cohort, United States, 1987–1992

Quartile (Q) of endogenous hormone	Model 1: minimally adjusted‡		Model 2: fully adjusted§	
	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval
Estrone (pg/ml)				
Q1 (11.52–25.23)	1.0	Reference	1.0	Reference
Q2 (25.29–32.32)	1.20	0.67, 2.15	1.05	0.54, 2.05
Q3 (32.33–41.20)	1.72	0.95, 3.09	1.26	0.62, 2.55
Q4 (41.27–154.78)	1.86	1.03, 3.36*	0.97	0.47, 2.01
DHEA-S (μg/dl)				
Q1 (5.68–48.4)	1.0	Reference	1.0	Reference
Q2 (48.43–72.80)	1.30	0.73, 2.33	1.17	0.60, 2.28
Q3 (72.88–107.61)	0.92	0.51, 1.64	0.74	0.37, 1.48
Q4 (107.79–497.40)	0.91	0.51, 1.64	0.68	0.35, 1.36
Androstenedione (ng/ml)				
Q1 (0.08–0.59)	1.0	Reference	1.0	Reference
Q2 (0.60–0.85)	1.49	0.83, 2.69	1.08	0.55, 2.12
Q3 (0.86–1.17)	1.58	0.88, 2.84	1.20	0.62, 2.32
Q4 (1.18–7.80)	1.59	0.88, 2.86	0.79	0.39, 1.60
Total testosterone (ng/dl)				
Q1 (2.50–12.4)	1.0	Reference	1.0	Reference
Q2 (12.5–20.94)	0.56	0.31, 1.01	0.60	0.30, 1.18
Q3 (20.97–36.1)	1.08	0.60, 1.95	1.00	0.50, 1.97
Q4 (36.2–462)	0.60	0.33, 1.09	0.34	0.16, 0.70**
Total testosterone/SHBG ratio				
Q1	1.0	Reference	1.0	Reference
Q2	0.70	0.40, 1.25	0.75	0.38, 1.46
Q3	0.76	0.42, 1.37	0.62	0.30, 1.24
Q4	0.98	0.54, 1.78	0.51	0.24, 1.10
SHBG (nmol/liter)				
Q1 (6.63–55.4)	1.0	Reference	1.0	Reference
Q2 (55.8–72.1)	0.49	0.26, 0.86*	0.52	0.27, 1.02
Q3 (72.2–95.79)	0.85	0.47, 1.53	0.95	0.47, 1.90
Q4 (95.84–244)	0.45	0.25, 0.82***	0.42	0.20, 0.90**

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.01$.

† DHEA-S, dehyroepiandrosterone sulfate; SHBG, sex hormone binding globulin.

‡ Adjusted for age and Atherosclerosis Risk in Communities (ARIC) center/race.

§ Simultaneously adjusted for age, ARIC field center/race, total cholesterol, high density lipoprotein cholestrol, systolic blood pressure, pack-years of smoking, sports index, fibrinogen, insulin, glucose, body mass index, and type of menopause.

appeared to be associated with a lower odds of carotid atherosclerosis. These relations were present after adjusting for multiple cardiovascular risk factors, including body mass index, thus suggesting an independent relation of these hormonal variables with carotid atherosclerosis. We found no associations of serum estrone, DHEA-S, and androstenedione with carotid atherosclerosis.

In univariate analysis, cases had significantly higher estrone and androstenedione levels but no difference in testosterone levels, which was not explained by differences in body mass index between the two groups. Cases did have significantly higher insulin levels. In polycystic ovary syndrome in premenopausal women, hyperinsulinemia is thought to stimulate androgen production by increasing luteinizing hormone secretion from the pituitary, enhancing the activity of ovarian and adrenal enzymes involved in steroid synthesis, and directly stimulating ovarian insulin receptors (37–39). This action might explain higher androstenedione and estrone (synthesized from the former) levels in cases prior to adjustment for insulin, although it is surprising that a similar relation was not found for testosterone. Under physiologic conditions, it is unclear whether insulin affects ovarian function (38). Analyses adjusting only for insulin attenuated the differences in hormone levels between cases and controls somewhat but did not account for the entire difference (data not shown), suggesting that multiple factors contribute to the association between hormones and atherosclerosis.

We found the lowest odds of carotid atherosclerosis for women with the highest total testosterone concentrations. In

the Rancho Bernardo Study, Barrett-Connor and Goodman-Gruen found no association between total or bioavailable testosterone and cardiovascular disease or ischemic heart disease death in postmenopausal women (40). Other studies have found associations of testosterone levels with increased levels of cardiovascular risk factors and risk of disease. Phillips et al. demonstrated a significant relation between free testosterone and angiographically determined coronary artery disease in one study (41) and between free testosterone and hypertension in postmenopausal women in another study (42).

Our findings are similar to those of Bernini et al., who found that postmenopausal women in the highest tertile of free testosterone had a significantly lower carotid intimal-medial thickness (26). In a study of young, healthy women without adrenal or ovarian disease, free testosterone was associated with a more favorable cardiovascular risk factor profile (43). Results of these two studies suggest that, at physiologic concentrations, testosterone may protect women against cardiovascular disease. In addition, testosterone is converted to estradiol, so that women with higher testosterone levels may also have higher estradiol levels (not measured in our study). Previous laboratory studies have suggested a role for androgens in vascular remodeling following injury (43, 44). A recent review suggests that androgen deficiency in postmenopausal women may even be a risk factor for cardiovascular disease (45). This suggestion is based on the observation that women who have undergone a premenopausal oophorectomy, and thus have had their major source of androgen production removed, have a higher incidence of cardiovascular disease compared with women who have not had this procedure, despite using hormone replacement (45).

In addition to testosterone, we found that higher SHBG levels were associated with a lower odds of carotid atherosclerosis. SHBG has been consistently found to be inversely related to many cardiovascular risk factors, including serum insulin (13), obesity (14, 15, 17, 46, 47), glucose tolerance (17), diastolic blood pressure (46), and triglycerides (47), and positively related to HDL cholesterol (13, 14, 46). Insulin is thought to be an important regulator of SHBG production (48). In vitro, insulin is a potent inhibitor of SHBG production by hepatoma cells, suggesting that decreased SHBG levels may indicate underlying insulin resistance (49). Insulin resistance, assessed by using the intravenous glucose tolerance test (50) and the euglycemic hyperinsulinemic clamp technique (51), has been found to be associated with increased asymptomatic atherosclerosis. Higher SHBG levels, if associated with lower insulin levels and less insulin resistance, would be expected to protect against atherosclerosis. Despite its association with a more favorable cardiovascular risk profile, SHBG has not been found to be associated with decreased cardiovascular mortality (20, 25).

There are two mechanisms by which SHBG may mediate its protective effect on atherosclerosis, apart from its association with a favorable risk profile: 1) by regulating levels of bioavailable androgen and estrogen or 2) by its direct effects at the cellular level. SHBG binds testosterone, dihydrotestosterone, and estradiol with high affinity, regulating their free concentrations (48). Because SHBG is stable and estradiol is bound with less affinity than testosterone, total estradiol is proportional to free estradiol (52). Therefore, higher SHBG levels may reflect higher endogenous estradiol concentrations, even in postmenopausal women, and hence may mediate estradiol's cardioprotective effect. In addition, Haffner et al. (16) hypothesized that SHBG may reflect intracellular bioavailable testosterone better than total testosterone, with reduced SHBG reflecting greater androgenicity. Finally, in vitro studies have demonstrated SHBG receptors in certain human tissue, which, when bound by SHBG and dihydrotestosterone or estradiol, increase intracellular cyclic adenosine monophosphate (48). Thus, in addition to transporting steroid hormones in plasma, SHBG may also help mediate the cardioprotective effects of estradiol and testosterone at the cellular level by regulating intracellular physiology.

We failed to find an association of DHEA-S, androstenedione, and estrone with carotid atherosclerosis. DHEA is converted to testosterone via androstenedione or Δ^5-androstenediol. Androstenedione is also converted to estrone, the principal estrogen in postmenopausal women (19). We found that DHEA-S and androstenedione are significantly correlated with testosterone and estrone (data not shown). Therefore, there may have been enough androgens and estrogens present that the protective effect of estrone was offset by the negative effects of DHEA-S and androstenedione, resulting in a lack of association.

In addition, a metabolite of DHEA, 5-androstene-3β, 17β-diol (ADIOL), has androgen- and estrogen-like effects (53). In postmenopausal women, ADIOL binding to estrogen receptors enhances the androgenic effects, which is less counterbalanced by the lower estrogen levels found in post- than in premenopausal women (53). DHEA-S, which is measured in epidemiologic studies (54), may not reflect ADIOL levels, which may more accurately reflect androgenicity. It is also thought that DHEA is more active than DHEA-S at the tissue level (53). Although one previous study showed no relation between DHEA-S level and cardiovascular risk factors in postmenopausal women (46), other studies have demonstrated positive associations of DHEA-S (55) and androstenedione (47) with cardiovascular risk factors. Higher DHEA-S concentrations have also been associated with a greater prevalence of cardiovascular disease (53). In contrast, Bernini et al. found that higher DHEA-S and androstenedione levels were protective for carotid atherosclerosis, independent of cardiovascular risk factors (26). With the exception of one study in which lower levels of DHEA-S were predictive of ischemic heart disease mortality in women (25), most studies have not shown a relation of androstenedione and DHEA-S levels to risk of death from cardiovascular disease (20, 40, 55).

Our findings support a previous study by Cauley et al., in which serum estrone concentrations were found not to be different between women with angiographically documented coronary artery disease and those without coronary artery disease (24). While lower levels of estradiol have been associated with an atherogenic lipid profile (47), no association has been found between estrone and estradiol

levels and ischemic heart disease mortality in postmenopausal women (20, 25). Our failure to observe a relation between serum estrone levels and carotid atherosclerosis would be expected if serum hormone levels do not correlate positively and linearly with intracellular levels.

Our study has three important strengths. It is the first known case-control study of the association between endogenous hormones and carotid atherosclerosis in postmenopausal women. Second, we were able to measure several hormonal variables and relate them to a clinically relevant outcome, subclinical atherosclerosis. While we have not presented data on cardiovascular events for this analysis, subclinical atherosclerosis, assessed by measuring intimal-medial thickness, has been shown to be predictive of incident coronary heart disease (56, 57). Third, the ARIC study has data on multiple cardiovascular risk factors, allowing an assessment of the independent association between each hormone and the odds of carotid atherosclerosis by using multivariate models.

A limitation of our study is that we did not measure free testosterone directly, as was done in more recent studies. However, our estimation of the total testosterone/SHBG ratio is a valid estimation of free testosterone and androgenicity (29). We did not measure estradiol, which is a more potent estrogen than estrone. Another problem is that, because the interassay coefficients of variation for estrone and androstenedione were >10 percent, there may have been random misclassification of hormone status, resulting in underestimation of odds ratios and failure to demonstrate an association between these hormones and carotid atherosclerosis. Furthermore, although thyroxine is a major regulator of SHBG, and SHBG levels can be elevated in hyperthyroidism, thyroid function was not measured in our study.

An additional limitation is that, because of limited power, we were unable to stratify our analysis by natural versus surgical menopause. The majority of the women in our sample underwent natural menopause, in which androgen and estradiol levels decline gradually as opposed to abruptly (58). If the rate of change in hormone levels or the ratio of estrogens to androgens is more important than the absolute hormone levels to the development of carotid atherosclerosis in postmenopausal women, then we would be unable to detect this relation by using our data. In addition, we were unable to adjust for time since onset of menopause and therefore could not account for the role that androgen deficiency may play in the development of carotid atherosclerosis over time following menopause. Finally, our data are cross-sectional, which limited our ability to infer a causal relation between carotid atherosclerosis and endogenous postmenopausal hormone levels. However, analyses to account for changes in health behavior did not alter our associations, making it less likely that health-related lifestyle changes accounted for hormone differences between cases and controls.

Our study has several implications. First, it suggests that estrogen levels may not be as important as testosterone levels with regard to risk of atherosclerosis in postmenopausal women not using hormone replacement therapy. In fact, testosterone deficiency in the postmenopausal state may enhance cardiovascular disease risk. Second, this study confirms previous findings of increased SHBG levels being associated with reduced cardiovascular disease risk, making it a potential marker for favorable cardiovascular outcomes. Finally, our data support further exploration of the potential benefit of adding androgen therapy to hormone replacement therapy in postmenopausal women with androgen deficiency as a way to modify cardiovascular outcomes.

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