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Endogenous Sex Hormones and Incident Cardiovascular Disease in Post-Menopausal Women



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ABSTRACT

BACKGROUND Higher androgen and lower estrogen levels are associated with cardiovascular disease (CVD) risk factors in women. However, studies on sex hormones and incident CVD events in women have yielded conflicting results.

OBJECTIVES The authors assessed the associations of sex hormone levels with incident CVD, coronary heart disease (CHD), and heart failure (HF) events among women without CVD at baseline.

METHODS The authors studied 2,834 post-menopausal women participating in the MESA (Multi-Ethnic Study of Atherosclerosis) with testosterone, estradiol, dehydroepiandrosterone, and sex hormone binding globulin (SHBG) levels measured at baseline (2000 to 2002). They used Cox hazard models to evaluate associations of sex hormones with each outcome, adjusting for demographics, CVD risk factors, and hormone therapy use.

RESULTS The mean age was 64.9 ± 8.9 years. During 12.1 years of follow-up, 283 CVD, 171 CHD, and 103 HF incident events occurred. In multivariable-adjusted models, the hazard ratio (95% confidence interval [CI]) associated with 1 SD greater log-transformed sex hormone level for the respective outcomes of CVD, CHD, and HF were as follows: total testosterone: 1.14 (95% CI: 1.01 to 1.29), 1.20 (95% CI: 1.03 to 1.40), 1.09 (95% CI: 0.90 to 1.34); estradiol: 0.94 (95% CI: 0.80 to 1.11), 0.77 (95% CI: 0.63 to 0.95), 0.78 (95% CI: 0.60 to 1.02); and testosterone/estradiol ratio: 1.19 (95% CI: 1.02 to 1.40), 1.45 (95% CI: 1.19 to 1.78), 1.31 (95% CI: 1.01 to 1.70). Dehydroepiandrosterone and SHBG levels were not associated with these outcomes.

CONCLUSIONS Among post-menopausal women, a higher testosterone/estradiol ratio was associated with an elevated risk for incident CVD, CHD, and HF events, higher levels of testosterone associated with increased CVD and CHD, whereas higher estradiol levels were associated with a lower CHD risk. Sex hormone levels after menopause are associated with women's increased CVD risk later in life. (J Am Coll Cardiol 2018;71:2555–66) © 2018 by the American College of Cardiology Foundation.



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ABBREVIATIONS AND ACRONYMS

CHD	= coronary heart disease
CI	= confidence interval
CRP	= C-reactive protein
CVD	= cardiovascular disease
DHEA	= dehydroepiandrosterone
EF	= ejection fraction
eGFR	= estimated glomerular filtration rate
HF	= heart failure
HFpEF	= heart failure with preserved ejection fraction
HFREF	= heart failure with reduced ejection fraction
HR	= hazard ratio
HT	= hormone therapy
IL	= interleukin
IQR	= interquartile range
LV	= left ventricular
SHBG	= sex hormone binding globulin
WHR	= waist-hip ratio

Atherosclerotic cardiovascular disease (CVD), which includes coronary heart disease (CHD) and ischemic stroke, affects men and women differently. In women, the risk of CVD is much lower than in men until 50 years of age, but it rises dramatically after menopause (1). It has been hypothesized that lower levels of endogenous estrogens and higher endogenous androgens that occur as a consequence of the menopausal transition might mediate the increased CVD risk later in life in post-menopausal women (1,2). This theory has been supported by observational studies that demonstrate associations between higher androgen and lower estrogen levels with CVD risk factors in post-menopausal women, including elevated blood pressure, C-reactive protein (CRP), and insulin resistance (1,3-5).

Studies examining the associations between sex hormone levels and CVD events in post-menopausal women, however, have yielded conflicting results. Both high (6,7) and low (8,9) androgen levels have been associated with increased risk of CVD. With respect to estrogens, observational studies have demonstrated that estradiol levels in women were either not associated (7,10) or inversely associated with CVD events (6). As a result, the relationship of sex hormones with CVD events in post-menopausal women is still unclear.

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Studies of the association between sex hormone levels and clinical CVD events in post-menopausal women, however, have been limited by suboptimal measurement of CVD events (10), use of hospital-based study populations (9,11), small sample size (11), or short duration of follow-up (7). Therefore, we used data from the MESA (Multi-Ethnic Study of Atherosclerosis) to evaluate the association of sex hormone levels with incident CVD, CHD, and heart failure (HF) events over 12-year follow-up in a community-based population of post-menopausal women free of CVD at baseline.

METHODS

STUDY PARTICIPANTS. MESA is a prospective cohort study of 6,814 men and women of 4 race/ethnicities

(white, black, Hispanic, and Chinese) who were 45 to 84 years of age and free of CVD at the baseline visit (2000 to 2002) (12). Participants were recruited from 6 centers across the United States (Los Angeles, California; St. Paul, Minnesota; New York City, New York; Chicago, Illinois; Forsyth County, North Carolina; Baltimore, Maryland). The present analysis included all post-menopausal women who attended the baseline exam ($n = 3,087$). We then excluded 160 participants missing sex hormone measurements, 2 participants without information on CVD outcomes, and 91 participants missing other covariates. The final sample size was 2,834 post-menopausal women (Figure 1). The study was approved by the institutional review boards of all participating institutions, and written informed consent was obtained from all participants.

MEASUREMENT OF SEX HORMONES. Sex hormone concentrations were measured from fasting serum samples that were drawn at the baseline examination between 7:30 AM and 10:30 AM and stored at -70°C until analysis. Biochemical analyses were performed at the University of Massachusetts Medical Center Sex Hormone Laboratory (Worcester, Massachusetts). Total testosterone and dehydroepiandrosterone (DHEA) were measured using radioimmunoassay kits. Sex hormone binding globulin (SHBG) was measured using a chemiluminescent enzyme immunometric assay (Immulite kits, Diagnostic Products Corporation, Los Angeles, California). Estradiol was measured using an ultrasensitive radioimmunoassay kit (Diagnostic System Laboratories, Webster, Texas). Concentrations of bioavailable testosterone (the sum of SHBG-bound and albumin-bound testosterone) and free testosterone (reported as a percentage of total testosterone) were calculated according to the method of Södergård (13). We divided total testosterone by estradiol to calculate total testosterone/estradiol ratio for each participant. Quality control serum was obtained from an $\sim 10\%$ blind pool. The coefficients of variation for total testosterone, SHBG, DHEA, and estradiol were 12.3%, 9.0%, 11.2%, and 10.5%, respectively (4).

CVD, CHD, AND HF ASSESSMENT. Every 9 to 12 months, participants completed a telephone interview about interim hospital admissions, cardiovascular outpatient diagnoses and procedures, and deaths (12). Hospital records were obtained for 98% of reported hospitalized CVD events, and some medical record-based information was obtained for 95% of

received research funds from GE Healthcare. Dr. Michos has received an honorarium from Siemens Diagnostics for being a blinded events adjudicator in a clinical trial. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Puja K. Mehta, MD, served as Guest Editor for this paper.

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outpatient encounters. Two physicians independently reviewed and adjudicated events, and assigned incidence dates. Outcome events were identified through December 31, 2013.

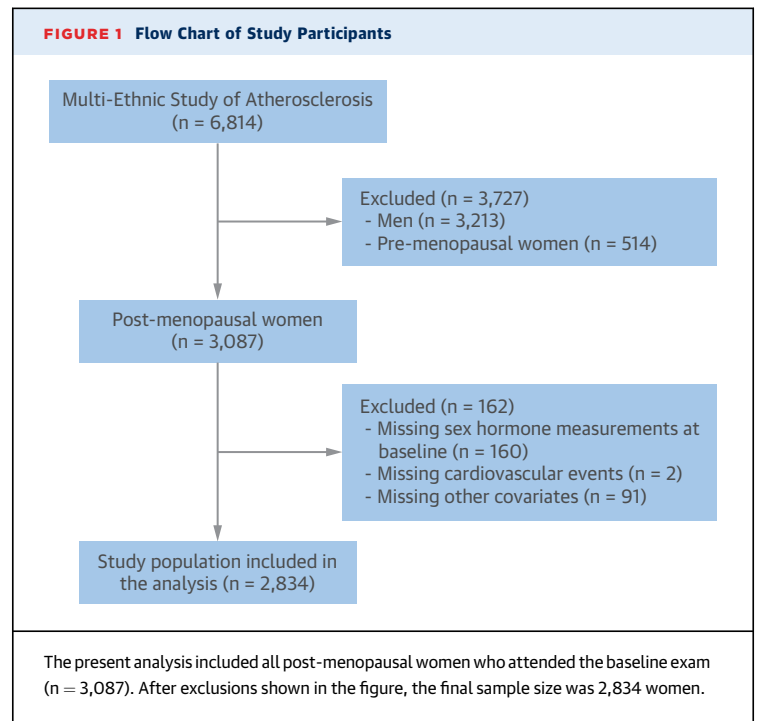
Incident CHD was defined as definite or probable myocardial infarction, resuscitated cardiac arrest, definite or probable angina (if followed by revascularization), and definite CHD death. Hard CHD events excluded definite or probable angina. Incident CVD events included incident CHD plus stroke, stroke death, other atherosclerotic death, and other CVD deaths. Hard CVD events included hard CHD, stroke, and stroke death. Ischemic stroke was determined by brain imaging with computed tomography or magnetic resonance imaging and included large vessel atherosclerosis, cardioembolism, small-vessel occlusion, and acute ischemic stroke of other unknown cause.

Incident HF was defined as definite or probable HF and required symptoms such as shortness of breath or edema. In addition to symptoms, probable HF required a HF diagnosis by a physician and medical treatment for HF. Definite HF required 1 or more criteria, such as pulmonary edema or congestion by chest x-ray, ventricular dilation or poor left ventricular (LV) function by echocardiography or ventriculography, or evidence of LV diastolic dysfunction. HF was classified as either HF with preserved ejection fraction (HFpEF) (LV ejection fraction [EF] $\geq 45\%$) or with reduced EF (HFrEF) (LVEF $< 45\%$) (14).

MEASUREMENT OF OTHER COVARIATES. Demographic characteristics, lifestyle factors, and medication use including hormone therapy (HT) were collected through standardized questionnaires. Menopausal status was self-reported and corrected using information on age, self-reported hysterectomy, bilateral oophorectomy, and menopausal age. Only post-menopausal women were included in this analysis.

Level of education was classified as <high school, high school/technical school/associate degree, and college/graduate/professional school. Physical activity was estimated as the total amount of intentional moderate or vigorous exercise performed in a usual week, and measured in metabolic equivalent task--minutes. Smoking status was categorized into never, former, or current smoker.

Height, weight, waist circumference, hip circumference, and blood pressure were measured by trained staff. Body mass index was calculated as weight in kilograms divided by height in meters squared. Waist-hip ratio (WHR) was calculated as waist circumference/hip circumference. Seated resting blood pressure was measured 3 times using an automated oscillometric sphygmomanometer (Dinamap Pro100, GE Healthcare, Milwaukee, Wisconsin),



and the average of the second and third measurements was used.

Blood samples were obtained from participants at the baseline examination after a 12-h fast. Details of measurements of clinical chemistry parameters are provided elsewhere (15). Diabetes was defined as a fasting serum glucose ≥ 126 mg/dl, a self-reported history of a physician diagnosis of diabetes, or use of insulin or diabetes medications. Estimated glomerular filtration rate (eGFR) was calculated on the basis of serum creatinine and cystatin C concentrations using the CKD-EPI equation (16). Inflammatory markers including CRP, interleukin-6 (IL-6), fibrinogen, and D-dimer were measured from stored samples, as previously described (17).

STATISTICAL ANALYSIS. Normally distributed continuous variables were expressed as mean \pm SD and compared by CVD status using the Student's *t*-test or analysis of variance. Variables with skewed distributions were expressed as median (interquartile range [IQR]) and compared using the nonparametric Wilcoxon rank sum test. Categorical variables were expressed as percentage (%) and compared using the chi-square test.

The associations between CVD risk factors and sex hormone levels were evaluated using multivariable-adjusted linear regression with \log_e -transformed sex hormone levels as dependent variables. The study endpoints were the development of incident CVD, CHD, or HF. Participants were followed from the

TABLE 1 Characteristics of Post-Menopausal Women at the MESA Baseline Exam (2000-2002) Followed for Incident CVD Through 2013				
Incident CVD status	Overall (N = 2,834)	No CVD (n = 2,551)	CVD (n = 283)	p Value
Age, yrs	64.9 ± 8.9	64.4 ± 8.9	69.3 ± 8.6	<0.001
Total T, nmol/l	0.90 (0.59-1.32)	0.90 (0.59-1.32)	0.94 (0.59-1.39)	0.06
Bioavailable T, nmol/l	0.21 (0.10-0.35)	0.21 (0.10-0.35)	0.24 (0.14-0.38)	0.04
Estradiol, nmol/l	0.073 (0.048-0.160)	0.073 (0.048-0.170)	0.066 (0.044-0.110)	0.05
Total T/estradiol ratio	11.82 (4.88-22.21)	11.55 (4.79-22.06)	15.42 (6.65-25.66)	0.001
DHEA, nmol/l	10.20 (6.97-14.50)	10.31 (6.97-14.71)	9.30 (6.25-13.05)	0.003
Free T, %	1.29 (0.89-1.70)	1.28 (0.88-1.70)	1.31 (0.90-1.70)	0.45
SHBG, nmol/l	59.50 (40.60-94.60)	59.85 (40.70-95.50)	58.10 (40.40-89.40)	0.38
Race/ethnicity				0.16
White	1,087 (38.4)	962 (37.7)	125 (44.2)	
Chinese-American	344 (12.1)	317 (12.4)	27 (9.5)	
Black	781 (27.6)	708 (27.8)	73 (25.8)	
Hispanic	622 (21.9)	564 (22.1)	58 (20.5)	
Education				<0.001
<High school	619 (21.9)	541 (21.2)	78 (27.6)	
High school, technical school, or associate degree	1,428 (50.4)	1,274 (50.0)	154 (54.4)	
College, graduate or professional school	785 (27.7)	734 (28.8)	51 (18.0)	
Smoking				0.14
Never	1,673 (59.1)	1,516 (59.5)	157 (55.5)	
Former	853 (30.1)	767 (30.1)	86 (30.4)	
Current	306 (10.8)	266 (10.4)	40 (14.1)	
Total intentional exercise, MET-min/week	3,540 (1,755-6,510)	3,630 (1,803-6,570)	2,925 (1,305-6,030)	0.004
Waist-to-hip ratio	0.91 (0.1)	0.91 (0.1)	0.94 (0.1)	<0.001
BMI, kg/m ²	28.6 (6.0)	28.5 (6.0)	29.6 (6.2)	0.002
Systolic BP, mm Hg	129.5 ± 23.5	128.4 ± 23.2	139.7 ± 23.7	<0.001
Diastolic BP, mm Hg	69.1 ± 10.3	68.9 ± 10.2	70.3 ± 11.1	0.03
Total cholesterol, mg/dl	201.5 ± 35.7	201.7 ± 35.6	199.5 ± 36.6	0.32
HDL cholesterol, mg/dl	56.7 ± 15.4	57.0 ± 15.5	54.0 ± 13.7	0.002
LDL cholesterol, mg/dl	118.4 ± 32.1	118.4 ± 32.2	118.5 ± 31.8	0.99
Triglycerides, mg/dl	131.7 ± 79.0	131.5 ± 80.2	133.3 ± 68.1	0.72
eGFR, ml/min/1.73 m ²	75.3 ± 15.7	75.8 ± 15.6	71.5 ± 16.8	<0.001
CRP, mg/l	2.6 (1.1-5.6)	2.5 (1.1-5.5)	3.2 (1.2-7.2)	0.02
D-dimer, µg/ml	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.3 (0.2-0.6)	<0.001
Fibrinogen, mg/dl	356 (311-405)	354 (309-403)	371 (326-432)	<0.001
IL-6, pg/ml	1.3 (0.8-2.0)	1.3 (0.8-1.9)	1.6 (1.0-2.5)	<0.001
Hormone therapy	900 (32.5)	829 (33.2)	71 (25.7)	0.01
Hypertension medication	1,179 (41.6)	1,010 (39.6)	169 (59.7)	<0.001
Lipid-lowering medication	532 (18.8)	463 (18.2)	69 (24.5)	0.01
Diabetes	349 (12.3)	285 (11.2)	64 (22.6)	<0.001
Values are mean ± SD, median (interquartile range), or n (%). To convert total, HDL, and LDL cholesterol from mg/dl to mmol/l, divide by 38.67. To convert total testosterone, free testosterone, bioavailable testosterone DHEA from nmol/l to ng/dl, divide by 0.0347. To convert estradiol from nmol/l to ng/ml, divide by 3.67. To convert SHBG from nmol/l to mg/l, divide by 10.5263. BMI = body mass index; BP = blood pressure; CRP = C-reactive protein; CVD = cardiovascular disease; DHEA = dehydroepiandrosterone; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; IL = interleukin; LDL = low-density lipoprotein; MESA = Multi-Ethnic Study of Atherosclerosis; MET-min = metabolic equivalent task-minute; SHBG = sex hormone binding globulin; T = testosterone.				

baseline visit until the development of a study endpoint, death, drop-out, or until December 31, 2013. Cox proportional hazards regression was used to estimate multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CIs) for each study endpoint associated with 1 SD greater log_e-transformed sex hormone levels. We also evaluated the presence of nonlinear dose-response relationships using restricted cubic splines with knots at the 5th, 35th, 65th, and 95th percentiles of their sample distributions.

We used 3 models with increasing degrees of adjustment. Model 1 adjusted for demographic factors: age, race/ethnicity, study site, and HT use. Model 2 further adjusted for lifestyle variables: education, WHR, physical activity, and smoking. Model 3 further adjusted for intermediate CVD risk factors: systolic blood pressure, use of

TABLE 2 HR (95% CI) for All CVD, CHD, and HF Associated With Sex Hormone Levels in Post-Menopausal Women: MESA 2000 to 2013

	HR (95% CI)		
	Model 1	Model 2	Model 3
All CVD (events n/N total = 283/2,834; IR = 9.3 per 1,000 person-yrs)			
Total T, nmol/l	1.17 (1.04-1.32)	1.15 (1.02-1.29)	1.14 (1.01-1.29)
Bioavailable T, nmol/l	1.17 (1.03-1.33)	1.10 (0.97-1.25)	1.07 (0.94-1.22)
Free T, %	1.11 (0.96-1.28)	0.99 (0.85-1.16)	0.92 (0.78-1.09)
Estradiol, nmol/l	1.03 (0.88-1.21)	0.99 (0.84-1.16)	0.94 (0.80-1.11)
Total T/estradiol ratio	1.13 (0.97-1.31)	1.15 (0.99-1.34)	1.19 (1.02-1.40)
DHEA, nmol/l	0.96 (0.85-1.09)	0.93 (0.82-1.04)	0.91 (0.81-1.03)
SHBG, nmol/l	0.89 (0.77-1.03)	0.99 (0.85-1.15)	1.07 (0.91-1.25)
All CHD (N events/N total = 171/2,834; IR = 5.5 per 1,000 person-yrs)			
Total T, nmol/l	1.21 (1.04-1.41)	1.19 (1.02-1.38)	1.20 (1.03-1.40)
Bioavailable T, nmol/l	1.18 (1.01-1.39)	1.10 (0.93-1.30)	1.08 (0.92-1.28)
Free T, %	1.07 (0.89-1.29)	0.94 (0.77-1.14)	0.85 (0.69-1.05)
Estradiol, nmol/l	0.90 (0.74-1.10)	0.84 (0.69-1.03)	0.77 (0.63-0.95)
Total T/estradiol ratio	1.28 (1.06-1.55)	1.33 (1.09-1.61)	1.45 (1.19-1.78)
DHEA, nmol/l	0.95 (0.81-1.11)	0.91 (0.78-1.06)	0.92 (0.78-1.07)
SHBG, nmol/l	0.93 (0.77-1.11)	1.06 (0.87-1.28)	1.17 (0.95-1.44)
All HF (events n/N total = 103/2,834; IR = 3.3 per 1,000 person-yrs)			
Total T, nmol/l	1.12 (0.92-1.36)	1.09 (0.90-1.33)	1.09 (0.90-1.34)
Bioavailable T, nmol/l	1.22 (0.99-1.50)	1.14 (0.92-1.41)	1.13 (0.90-1.41)
Free T, %	1.10 (0.86-1.40)	0.98 (0.76-1.27)	0.91 (0.69-1.20)
Estradiol, nmol/l	0.90 (0.70-1.17)	0.87 (0.67-1.13)	0.78 (0.60-1.02)
Total T/estradiol ratio	1.20 (0.93-1.54)	1.21 (0.93-1.55)	1.31 (1.01-1.70)
DHEA, nmol/l	0.86 (0.71-1.05)	0.84 (0.69-1.02)	0.85 (0.70-1.04)
SHBG, nmol/l	0.91 (0.71-1.15)	1.00 (0.78-1.29)	1.09 (0.83-1.43)
HFpEF (events n/N total = 55/2,821; IR = 1.8 per 1,000 person-yrs)			
Total T, nmol/l	1.25 (0.96-1.64)	1.21 (0.92-1.58)	1.22 (0.91-1.62)
Bioavailable T, nmol/l	1.21 (0.91-1.61)	1.08 (0.80-1.46)	1.10 (0.80-1.51)
Free T, %	1.07 (0.76-1.49)	0.88 (0.62-1.26)	0.91 (0.62-1.33)
Estradiol, (nmol/l)	1.14 (0.79-1.66)	1.10 (0.75-1.62)	0.98 (0.67-1.43)
Total T/estradiol ratio	1.13 (0.80-1.60)	1.13 (0.79-1.61)	1.22 (0.85-1.75)
DHEA, nmol/l	1.18 (0.89-1.58)	1.15 (0.86-1.52)	1.21 (0.90-1.63)
SHBG, nmol/l	0.94 (0.67-1.30)	1.12 (0.79-1.60)	1.10 (0.75-1.60)
HFrEF (events n/N total = 35/2,821; IR = 1.1 per 1,000 person-yrs)			
Total T, nmol/l	1.10 (0.79-1.55)	1.09 (0.78-1.53)	1.11 (0.80-1.56)
Bioavailable T, nmol/l	1.24 (0.86-1.77)	1.20 (0.83-1.73)	1.19 (0.82-1.73)
Free T, %	1.11 (0.75-1.67)	1.07 (0.70-1.64)	1.01 (0.64-1.59)
Estradiol, (nmol/l)	0.65 (0.44-0.97)	0.64 (0.43-0.97)	0.60 (0.39-0.93)
Total T/estradiol ratio	1.54 (1.02-2.32)	1.52 (1.00-2.31)	1.65 (1.07-2.54)
DHEA, nmol/l	0.63 (0.48-0.81)	0.59 (0.45-0.77)	0.59 (0.44-0.78)
SHBG, nmol/l	0.90 (0.61-1.35)	0.94 (0.62-1.44)	1.00 (0.64-1.58)
All sex-hormones are log-transformed and standardized. Hazard ratios are associated with 1-SD increase in log sex hormones. Bold results are statistically significant. Model 1 adjusts for age (year, continuous), race/ethnicity (white, black, Hispanic, and Chinese), study site (California, Minnesota, New York, Illinois, North Carolina, and Maryland), and use of hormone therapy (yes, no). Model 2 adjusts for model 1 variables + education (<high school, high school, >high school), waist-to-hip ratio (continuous), physical activity, and smoking (never, former, current). Model 3 adjusts for model 2 variables + systolic blood pressure (mm Hg, continuous), use of antihypertensive medications (yes, no), total cholesterol (mg/dl, continuous), HDL cholesterol (mg/dl, continuous), use of lipid-lowering medications (yes, no), diabetes (yes, no), eGFR (mL/min/1.73 m ²), CRP (mg/L, log-transformed continuous), IL-6 (pg/mL, log-transformed, continuous), fibrinogen (mg/dL, log-transformed continuous), D-dimer (μg/mL, log-transformed continuous).			
CHD = coronary heart disease; CI = confidence interval; HF = heart failure; HR = hazard ratio; IR = incidence rate; other abbreviations as in Table 1.			

antihypertensive medications, total cholesterol, high-density lipoprotein cholesterol, use of lipid lowering medication, diabetes, eGFR, CRP, IL-6, fibrinogen, and D-dimer. In the primary analyses,

the associations between sex hormones and each of the CVD endpoints were modeled separately for each individual hormone. In secondary analyses, we included total testosterone, estradiol, DHEA, and

TABLE 3 HRs (95% CI) for All CVD, CHD, and HF Excluding Women on HT (N = 1,934)

	HR (95% CI)		
	CVD (n = 212, IR = 10.3)	CHD (n = 127, IR = 6.1)	HF (n = 81, IR = 3.9)
Total T, nmol/l	1.13 (0.99-1.31)	1.19 (0.99-1.43)	1.17 (0.93-1.48)
Bioavailable T, nmol/l	1.09 (0.93-1.29)	1.07 (0.87-1.31)	1.19 (0.91-1.56)
Free T, %	0.86 (0.69-1.08)	0.71 (0.54-0.93)	0.77 (0.54-1.09)
Estradiol, nmol/l	0.97 (0.78-1.19)	0.76 (0.58-0.99)	0.75 (0.54-1.04)
Total T/estradiol ratio	1.21 (0.99-1.48)	1.55 (1.20-2.00)	1.51 (1.10-2.06)
DHEA, nmol/l	0.98 (0.84-1.13)	1.01 (0.83-1.22)	0.88 (0.69-1.12)
SHBG, nmol/l	1.14 (0.93-1.41)	1.39 (1.07-1.82)	1.25 (0.89-1.75)

All sex-hormones are log-transformed and standardized. HRs are associated with 1-SD increase in log sex hormones. **Bold** results are statistically significant. Model adjusts for age (year, continuous), race/ethnicity (white, black, Hispanic, and Chinese), and study site (California, Minnesota, New York, Illinois, North Carolina, and Maryland), education (<high school, high school, >high school), waist-to-hip ratio (continuous), physical activity, and smoking (never, former, current), systolic blood pressures (mm Hg, continuous), use of antihypertensive medications (yes, no), total cholesterol (mg/dL, continuous), HDL cholesterol (mg/dL, continuous), use of lipid-lowering medications (yes, no), diabetes (yes, no), eGFR (mL/min/1.73 m²), CRP (mg/L, log-transformed continuous), IL-6 (pg/mL, log-transformed continuous), fibrinogen (mg/dL, log-transformed continuous), D-dimer (μg/mL, log-transformed continuous).

HT = hormone therapy; other abbreviations as in [Tables 1 and 2](#).

SHBG in the fully adjusted model to mutually account for one another.

We performed stratified analyses in pre-specified subgroups defined by age and race/ethnicity. We also performed sensitivity analyses excluding women who were using HT at baseline (because estradiol levels differ substantially among women taking vs. not taking HT), and using hard CVD and hard CHD events as endpoints instead of all CVD and CHD events. Additionally, we conducted analysis using non-CVD death as a competing risk. All reported p values were 2-sided, and the significance level was set at 0.05. Statistical analyses were performed using STATA version 14 (StataCorp LP, College Station, Texas).

RESULTS

The baseline characteristics of the study population are presented in [Table 1](#). The average age at baseline was 64.9 ± 8.9 years (IQR: 58 to 72 years). The median (IQR) of sex hormone levels in nmol/L were: total testosterone 0.90 (IQR: 0.59 to 1.32), estradiol 0.07 (IQR: 0.05 to 0.16), DHEA 10.20 (IQR: 6.97 to 14.50), and SHBG 59.50 (IQR: 40.60 to 94.60), and for testosterone/estradiol ratio was 11.82 (IQR: 4.88 to 22.21). The demographic-adjusted associations of CVD risk factors (independent variables) with sex hormone levels (dependent variables) at the baseline examination are presented in [Online Table 1](#).

Current smoking was associated with higher total testosterone, testosterone/estradiol ratio, and DHEA levels, and lower estradiol levels. Obesity and CRP were associated with higher total testosterone and estradiol, and with lower testosterone/estradiol ratio and SHBG levels.

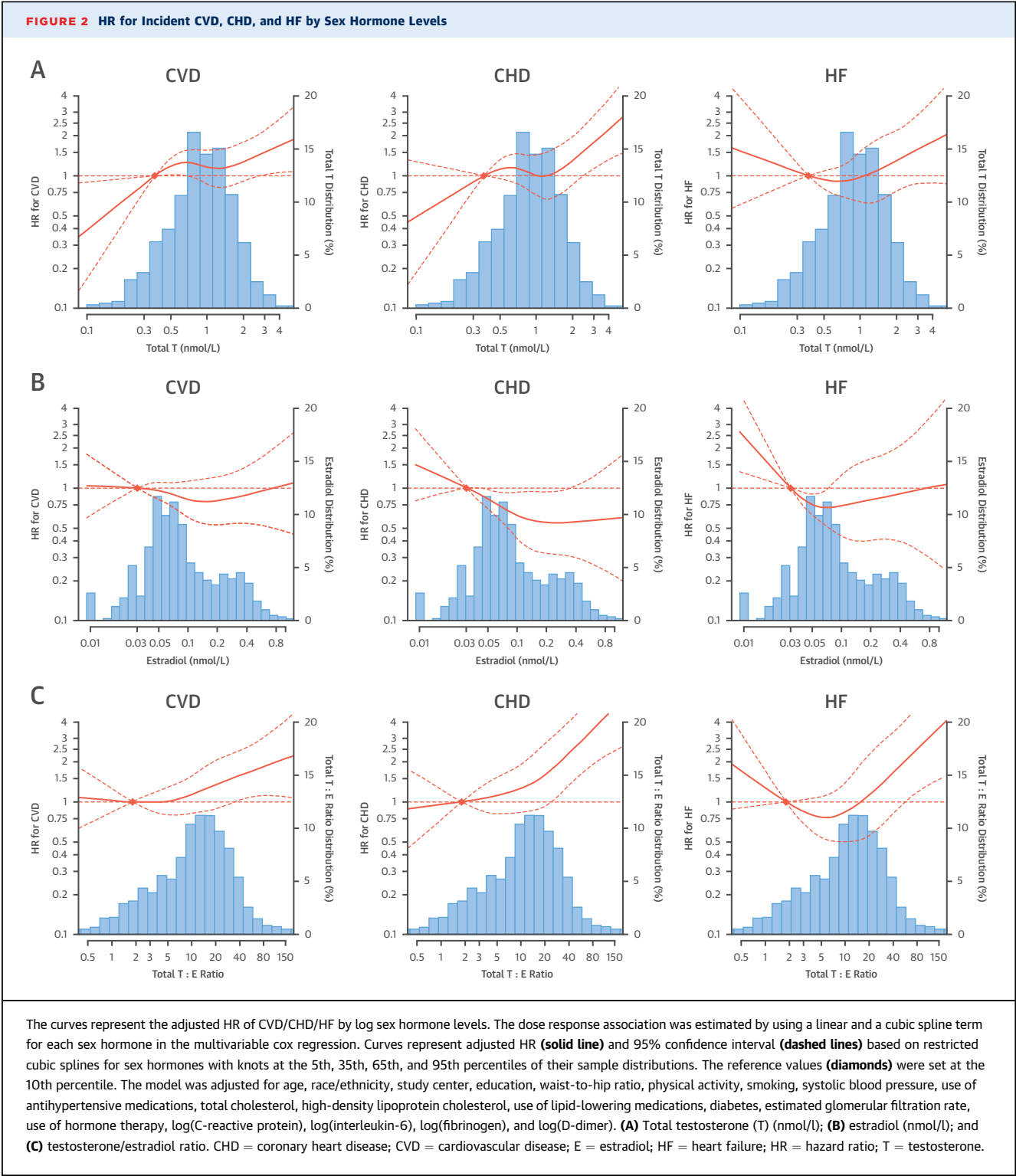
During 30,487 person-years of follow-up (median [IQR] follow-up of 12.1 [IQR: 11.5 to 12.7] years), the numbers of women who developed incident CVD, CHD, and HF events were 283, 171, and 103, respectively (incidence rates of 9.3, 5.5, and 3.3 per 1,000 person-years, respectively). At baseline, women who developed incident CVD were more likely to be older, diabetic, hypertensive, have higher WHR, CRP, D-dimer, fibrinogen, IL-6, bioavailable testosterone levels, and testosterone/estradiol ratio, and have lower education, DHEA and high-density lipoprotein cholesterol levels, eGFR, and physical activity ([Table 1](#)).

A higher total testosterone/estradiol ratio was independently associated with an increased risk of incident CVD, CHD, and HF ([Table 2](#)). The fully adjusted HRs for CVD, CHD, and HF associated with 1 SD greater log-transformed testosterone/estradiol ratio was 1.19 (95% CI: 1.02 to 1.40), 1.45 (95% CI: 1.19 to 1.78), and 1.31 (95% CI: 1.01 to 1.70), respectively (model 3). Total testosterone levels were also associated with an increased risk of incident CVD and CHD, but were not statistically significantly associated with HF. The corresponding HRs associated with 1 SD increase in log-transformed total testosterone were 1.14 (95% CI: 1.01 to 1.29), 1.20 (95% CI: 1.03 to 1.40), and 1.09 (95% CI: 0.90 to 1.34), respectively. Bioavailable testosterone was associated with CVD and CHD in demographic-adjusted models, but the associations were attenuated to null in fully-adjusted models. The corresponding HRs per 1 SD greater testosterone/estradiol ratio for the other endpoints were directionally consistent with the primary analyses as follows: hard CVD 1.29 (95% CI: 1.07 to 1.56), hard CHD 1.57 (95% CI: 1.22 to 2.02), and ischemic stroke 1.10 (95% CI: 0.81 to 1.48) ([Online Table 2](#)) (model 3).

Estradiol levels were not associated with overall CVD events, but were associated with a lower risk for CHD, with a non-significant trend for lower HF risk. The fully adjusted HRs for CVD, CHD, and HF associated with 1 SD greater log-transformed estradiol were 0.94 (95% CI: 0.80 to 1.11), 0.77 (95% CI: 0.63 to 0.95) and 0.78 (95% CI: 0.60 to 1.02), respectively ([Table 2](#)) (model 3). The corresponding HRs per 1 SD greater estradiol for other endpoints were as follows: hard CVD 0.88 (95% CI: 0.72 to 1.06), hard CHD 0.69 (95% CI: 0.53 to 0.88), and ischemic stroke 1.11 (95% CI: 0.80 to 1.55) ([Online Table 2](#)) (model 3).

DHEA and SHBG levels were not associated with CVD, CHD, all HF, hard CVD, hard CHD, or ischemic stroke in primary analyses.

Regarding the HF subtypes, testosterone/estradiol ratio was positively associated, and both estradiol

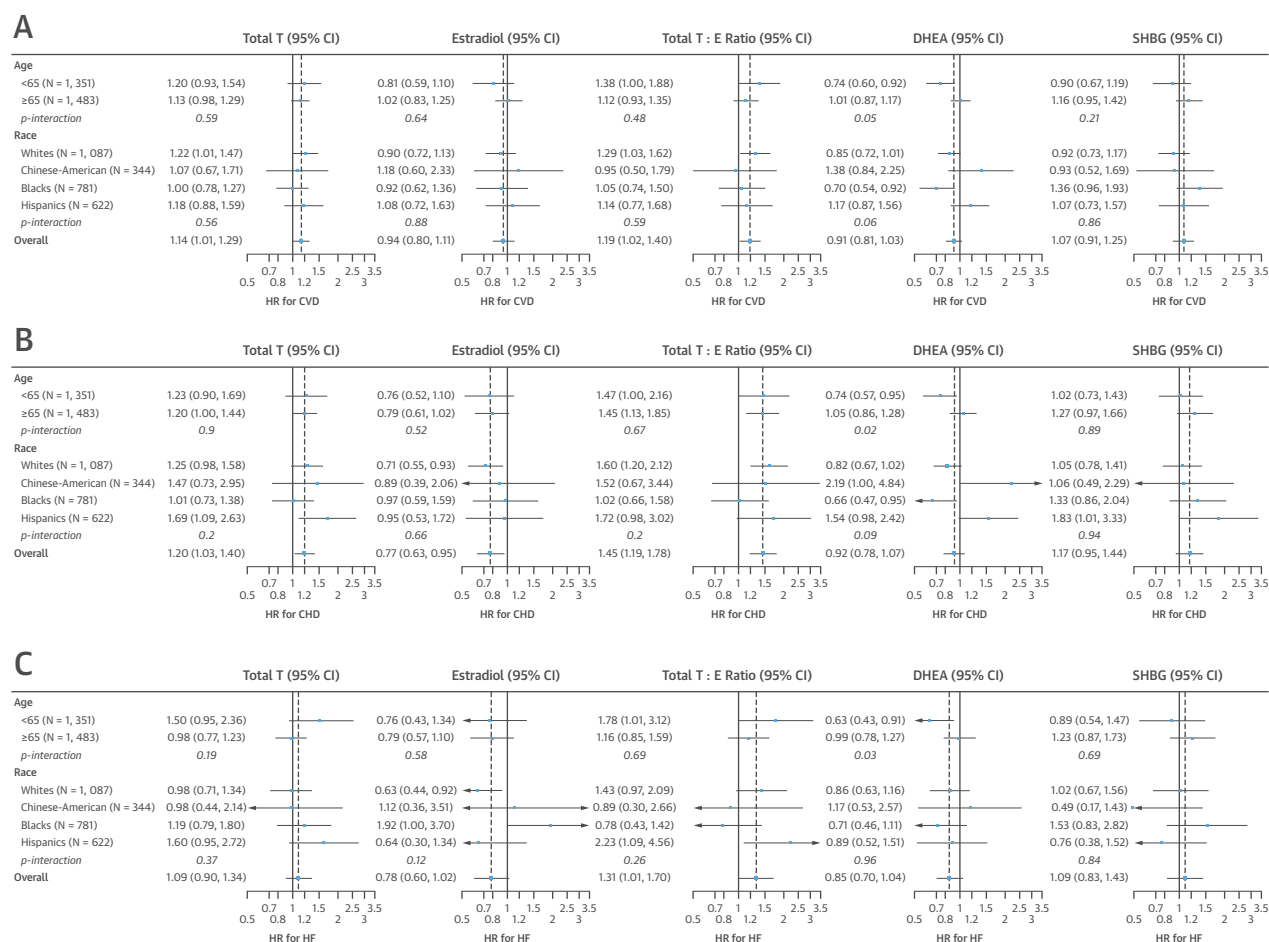


and DHEA inversely associated, with HFrEF, but not HFpEF (Table 2). The corresponding HRs for HFrEF per 1 SD greater sex hormone level were: testosterone/estradiol 1.65 (95% CI: 1.07 to 2.54), estradiol

0.60 (95% CI: 0.39 to 0.93), and DHEA 0.59 (95% CI: 0.44 to 0.78).

In sensitivity analyses excluding women taking HT (Table 3), testosterone/estradiol ratio remained

FIGURE 3 Associations Between Sex Hormone Levels With Incident CVD, CHD, and HF by Age and Race/Ethnic Subgroups



(A) HR for CVD; **(B)** HR for CHD; and **(C)** HR for HF. *HRs are associated with 1 SD increase in log sex hormones. The model was adjusted for the covariates listed for [Figure 2](#). DHEA = dehydroepiandrosterone; SHBG = sex hormone binding globulin; other abbreviations as in [Figure 2](#).

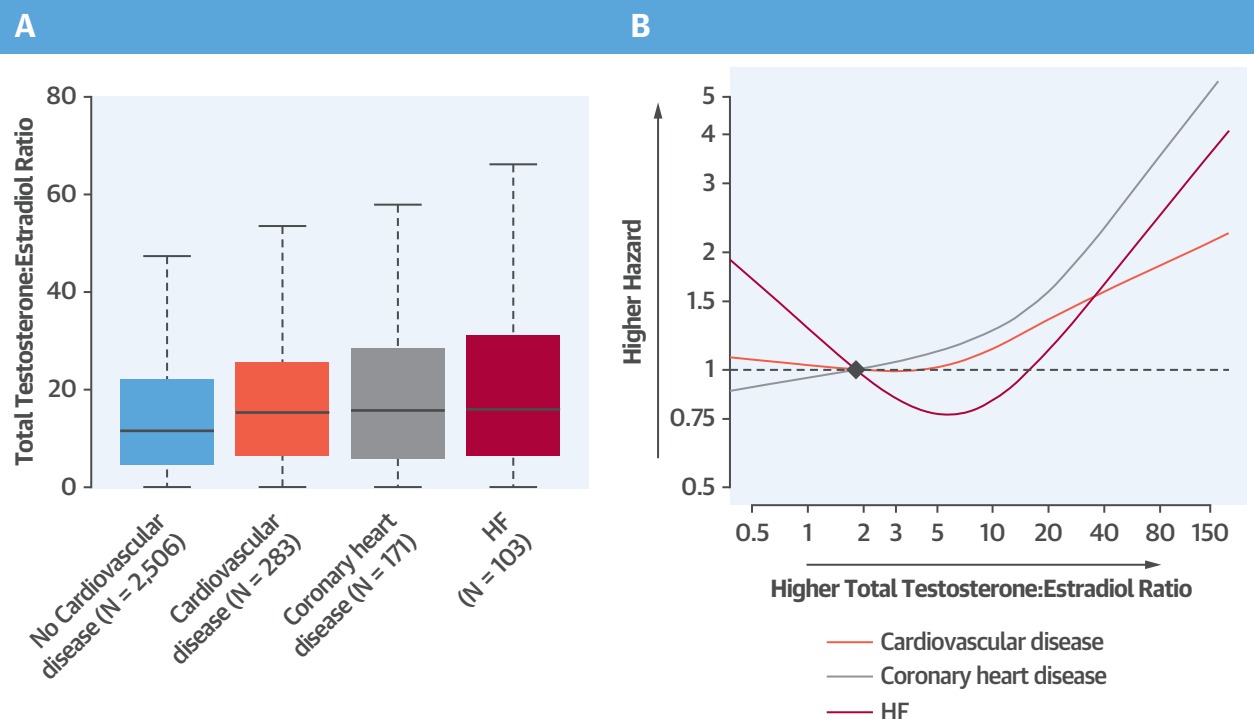
positively associated with CHD and HF, whereas estradiol was inversely associated with CHD, similar to the primary analyses. However, in this sensitivity analysis, free testosterone was now inversely associated with CHD (0.71 [95% CI: 0.54 to 0.93]) and SHBG levels were positively associated with CHD (1.39 [95% CI: 1.07 to 1.82]). When all sex hormones were included in the same model, the associations remained similar ([Online Table 3](#) for all women and [Online Table 4](#) for women not on HT). Additional competing risk analyses yielded similar results to primary analysis ([Online Table 5](#)).

In spline regression analyses, the risk of incident CVD and CHD increased progressively with total testosterone levels and testosterone/estradiol ratio ([Figures 2A and 2C](#)), and the risk of incident CHD and HF decreased progressively with estradiol levels

([Figure 2B](#)), adjusted for variables in model 3. The *p* values for the nonlinear spline components of total testosterone for CVD, CHD, and HF were 0.17, 0.08, and 0.22, respectively; for testosterone/estradiol ratio were 0.40, 0.13, and 0.006, respectively; and for estradiol were 0.58, 0.49, and 0.06, respectively, indicating that the associations of sex hormones with these endpoints were approximately linear, except for testosterone/estradiol ratio and risk of HF, which was U-shaped.

The associations between sex hormones and CVD outcomes were generally consistent across all age and race/ethnic subgroups, except that in younger participants (<65 years of age), the association of DHEA levels with CVD, CHD, and HF endpoints was inverse whereas it was null in older participants (≥65 years) ([Figure 3](#)).

CENTRAL ILLUSTRATION Testosterone/Estradiol Ratio and the Risk of Incident CVD, CHD, and HF in Post-Menopausal Women: MESA



Zhao, D. et al. J Am Coll Cardiol. 2018;71(22):2555-66.

(A) Boxplot distribution of the testosterone/estradiol ratio by outcome status (unadjusted). The **bottom and top of the box** are the first and third quartiles, and the **band inside the box** is the second quartile (the median). The **whiskers** represent the first quartile $-1.5 \times$ interquartile range and 3rd quartile $+1.5 \times$ interquartile range. (B) Adjusted hazard ratio of CVD/CHD/HF for testosterone/estradiol ratio using restricted cubic spline with knots at the 5th, 35th, 65th, and 95th percentiles of the sample distribution. The reference value (**diamond**) was set at the 10th percentile. The model was adjusted for age, race/ethnicity, study center, use of hormone therapy, education, waist-to-hip ratio, physical activity, smoking, systolic blood pressure, use of antihypertensive medications, total cholesterol, high-density lipoprotein cholesterol, use of lipid lowering medications, diabetes, estimated glomerular filtration rate, log(C-reactive protein), log(interleukin-6), log(fibrinogen), and log(D-dimer). CHD = coronary heart disease; CVD = cardiovascular disease; HF = heart failure; MESA = Multi-Ethnic Study of Atherosclerosis.

DISCUSSION

Among racially/ethnically diverse post-menopausal women followed for >12 years, we found that higher testosterone/estradiol ratio was associated with an elevated risk for incident CVD, CHD, and HF events, higher total testosterone was associated with increased risk of CVD and CHD, and higher estradiol levels were associated with a lower risk of CHD (Central Illustration). The associations persisted after adjustment for traditional cardiovascular risk factors, suggesting an independent role of sex hormones on cardiovascular events. The risks for CVD and CHD were approximately linear across the whole range of total testosterone, testosterone/estradiol ratio, and estradiol levels. However, there

was a U-shaped association between testosterone/estradiol ratio and HF, with both extreme ends at higher risk for HF. Additionally, we found that testosterone/estradiol ratio was positively, and estradiol and DHEA were inversely, associated with risk of HFrEF.

Several biological mechanisms may underlie the association between endogenous sex hormones and CVD and its risk factors in women. Estrogens can promote vasodilation through increasing plasma concentrations of endothelium-derived relaxing factor nitric oxide, and can inhibit the renin angiotensin system by reducing transcription of angiotensin-converting enzyme (18). In addition to its favorable effects on lipids, estrogens can also reduce blood pressure through increasing endothelial vasodilator

function and modulating autonomic function. Additionally, estrogen is thought to regulate specific inflammatory markers and cytokines (19). By contrast, testosterone can induce vasoconstriction and increased platelet aggregation through up-regulation of thromboxane (20). In population studies, androgens are associated with increased accumulation of visceral fat, lipid levels, and increased levels of cardiometabolic risk factors (2,3,21), including blood pressure, body mass index, CRP, and insulin resistance in women (1,3-5). Furthermore, a history of early age of menopausal onset and shorter duration from menarche to menopause are associated with higher risk of cardiovascular outcomes, including CVD mortality, CHD, and HF (22,23); this again suggests a role of sex hormones in disease pathogenesis.

Despite plausible pathological pathways linking endogenous sex hormones and clinical CVD events, prospective studies have been contradictory. In a population-based study in Denmark ($N = 4,600$ premenopausal and post-menopausal women), higher baseline total testosterone levels were associated with an increased risk of CHD and death over 30 years follow-up (6). In the WHS (Women's Health Study), 200 post-menopausal women who developed CVD >3 years of follow-up had a higher baseline free androgen index compared with controls, but the association was null after adjustment for CVD risk factors (7), and a population-based cohort study in Germany ($N = 2,129$ middle-aged women, 11-year follow-up) also concluded that baseline total testosterone was not associated with incident CVD or mortality (24). Furthermore, the Rancho Bernardo Study (8,10) ($N = 639$ post-menopausal women, 12-year follow-up) and a hospital-based cohort study in Germany (9) ($N = 2,914$ women between 18 and 75 years of age, 4.5-year follow-up) both found that lower baseline levels of total testosterone were associated with increased CVD events, which contrasts with our findings.

With respect to estrogens, a population-based study in Denmark found that lower estradiol levels were associated with increased risk of CHD and death (6), but the WHS trial (7), Rancho Bernardo Study (8,10), and a nested case-control study of elderly diabetic women (11) found no association between estradiol levels and CVD events.

Several methodological differences could explain the discrepancies in the associations observed in previous studies. First, previous studies have used different study designs, with a mixture of cohort (8,9,24), nested cohort (6), nested case-control designs (7), with follow-up times ranging from 4 to almost 30 years. Second, the study populations were

very diverse, with mean age ranging from 49 to 74 years. Finally, these studies used different measurements of CVD events, including self-reports (8,10,24), national patient registries (6), and medical records (7,9).

Our study supports an association of higher total testosterone levels and higher testosterone/estradiol ratio with increased CVD and CHD events in post-menopausal women, as well as an association of higher estradiol levels with reduced CHD and HF events, although the association between estradiol and HF did not reach statistical significance (HF events were fewer than CHD events, with therefore less power to detect associations). Among HF subtypes, we found that higher testosterone/estradiol ratio and lower estradiol levels were associated with increased risk for HFrEF, but not HFpEF, the type of HF more commonly seen in older women. It is possible that the reduction in estradiol during menopause differentially affects vascular and cardiac remodeling processes that differentially lead to a HFrEF versus HFpEF phenotype. Lower levels of estradiol are associated with risk for hypertension (25), a risk factor for HFrEF. In our study, estradiol was associated with CHD, and CHD may contribute more to HFrEF than to HFpEF. As mentioned, our results contradicted the 2 Germany studies and Rancho Bernardo Study that showed null or reversed association between total testosterone and CVD events (8,9,24). The difference may be due to the selection of study participants (younger women with mean age 49 years, and hospital-based in the German study) or ascertainment of CVD outcome (self-report in Rancho Bernardo Study). Further studies are needed to confirm these findings and to better understand the associations and mechanisms between sex hormones and subtypes of HF.

In our study, SHBG and DHEA levels were not associated with CVD, CHD, or overall HF. However, SHBG was associated with increased risk for CHD in women not taking HT. This result aligns with a previous MESA study analysis showing that SHBG was positively associated with coronary artery calcification (15). Nevertheless, other studies have not found an association between SHBG and incident CVD (7,10,11,24). Additionally, in our study, DHEA was inversely associated with HFrEF risk, an association that needs to be confirmed in other studies. The role of DHEA on CVD is still undefined. It was hypothesized that lower DHEA levels might be related to increased risk of CVD (26), but this association was refuted by many studies, especially among women (27,28).

Although sex hormone levels may be linked to future CVD events, it is unclear what the best

intervention is to modify sex hormone levels for risk reduction. Although randomized clinical trials failed to conclusively show a beneficial effect of HT on CHD risk or CVD mortality in post-menopausal women in primary prevention (29,30), the trials generally did not consider compounds other than conjugated equine estrogen and medroxyprogesterone acetate, and the conclusions may not be generalized to all menopausal women in different ages (29,31,32). HT may not exert the same physiological effects as endogenous sex hormones, and effects vary depending on the different formulations of estrogen and progestin (33). Also, the time of HT initiation may determine HT effects on CVD risk. It has been suggested that estrogen therapy may prevent subclinical atherosclerosis in perimenopausal women when given around the time of the menopausal transition, but has no benefit on CVD risk in later life after menopause (29,34,35).

STUDY STRENGTHS AND LIMITATIONS. Our study has many strengths, such as the inclusion of a large, diverse, well-characterized multiethnic group of women prospectively followed for more than 10 years. We investigated the associations of sex hormones with a variety of CVD outcomes after careful adjustment for a number of potential confounders and use of HT.

However, our results should be considered in the context of some limitations. First, despite the sample size and the long duration of follow-up, the number of events observed in our study was limited for some outcomes, particularly for HF subtypes and ischemic stroke, limiting our power to detect associations. Additionally, although large variation of associations across race/ethnicity were seen, the sample size may limit the detection of significant interaction between sex hormone levels and race/ethnicity. Second, we studied multiple sex hormones and outcomes, and some associations may have been observed by chance. However, we based our conclusions not only on significance tests, but also on biological plausibility, dose-response, and consistency of the associations. Third, although we adjusted for multiple potential confounders, we cannot exclude the possibility of residual confounding. Fourth, menopausal status was self-reported and subject to reporting bias, although we corrected self-reported menopausal status using an algorithm based on age, self-reported hysterectomy, bilateral oophorectomy, and menopausal age. Finally, sex hormone levels were only assessed once at baseline and were not repeatedly measured during follow-up. In particular,

we did not have information on sex hormone levels before and during the menopausal transition. As a consequence, we could not examine the association of changes in sex hormone levels with CVD events.

CONCLUSIONS

Our study suggests that, in post-menopausal women, higher testosterone/estradiol ratio is associated with the development of CVD, CHD, and HF events, whereas estradiol is associated with a reduced risk of CHD and HF. Sex hormone levels, especially higher total testosterone versus estrogen after menopause, may contribute to women's increased CVD risk later in life. However, in the absence of supportive interventional studies, the best strategy to modify sex hormone levels to affect CVD risk is still uncertain. Nonetheless, a more androgenic sex hormone profile may identify a woman at higher risk for CVD who may benefit from other risk-reducing strategies.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In post-menopausal women, a higher total testosterone/estradiol ratio was associated with increased risk for incident CVD, CHD, and HF. Total testosterone levels were associated with cardiovascular and coronary events, while estradiol was associated with a reduced risk of coronary events and HF.

TRANSLATIONAL OUTLOOK: Further studies are needed to identify individual patient risk factors that interact with sex hormones in menopausal women to guide development and implementation of preventive strategies.

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KEY WORDS cardiovascular disease, coronary heart disease, estradiol, heart failure, sex hormone binding globulin, sex hormones, testosterone

APPENDIX For supplemental tables, please see the online version of this paper.