

# Endogenous Sex Hormones and Cardiovascular Disease Incidence in Men

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**Background:** Data suggest that endogenous sex hormones (testosterone, dehydroepiandrosterone sulfate [DHEA-S], and estradiol) influence cardiovascular disease (CVD) risk factors and vascular function. Yet, prospective studies relating sex hormones to CVD incidence in men have yielded inconsistent results.

**Objective:** To examine the association of circulating sex hormone levels and CVD risk in men.

**Design:** Prospective cohort study.

**Setting:** Community-based study in Framingham, Massachusetts.

**Participants:** 2084 middle-aged white men without CVD at baseline.

**Measurements:** The authors used multivariable Cox regression to relate baseline levels of testosterone, DHEA-S, and estradiol to the incidence of CVD (coronary, cerebrovascular, or peripheral vascular disease or heart failure) during 10 years of follow-up.

**Results:** During follow-up, 386 men (18.5%) experienced a first CVD event. After adjustment for baseline standard CVD risk factors, higher estradiol level was associated with lower risk for CVD

(hazard ratio per SD increment in log estradiol, 0.90 [95% CI, 0.82 to 0.99];  $P = 0.035$ ). The authors observed effect modification by age: Higher estradiol levels were associated with lower CVD risk in older (median age >56 years) men (hazard ratio per SD increment, 0.86 [CI, 0.78 to 0.96];  $P = 0.005$ ) but not in younger (median age  $\leq 56$  years) men (hazard ratio per SD increment, 1.11 [CI, 0.89 to 1.38];  $P = 0.36$ ). The association of higher estradiol level with lower CVD incidence remained robust in time-dependent Cox models (updating standard CVD risk factors during follow-up). Serum testosterone and DHEA-S levels were not statistically significantly associated with incident CVD.

**Limitations:** Sex hormone levels were measured only at baseline, and the findings may not be generalizable to women and nonwhite people.

**Conclusions:** In the community-based sample, a higher serum estradiol level was associated with lower risk for CVD events in older men. The findings are consistent with the hypothesis that endogenous estrogen has vasculoprotective influences in men.

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Male sex is an independent risk factor for cardiovascular disease (CVD) (1). Scientists have postulated that the 5- to 10-year lag period in CVD incidence in women (compared with men) may be related to differences in endogenous sex hormones (2–7). Indeed, substantial evidence suggests that sex hormones (testosterone, estrogen, and dehydroepiandrosterone sulfate [DHEA-S]) influence traditional and newer CVD risk factors (2–7). Interest in the role of sex hormones in the pathogenesis of CVD has been rekindled by the observation that men with genetic defects of estrogen synthesis (8) or action (9) develop premature atherosclerosis. In addition, genetic variation in estrogen receptor- $\alpha$  has been associated with prevalent CVD (10, 11), and androgen and estrogen receptor expression in coronary arteries has been reported to influence coronary atherosclerosis in men (12).

In contrast to the aforementioned data, prospective studies relating circulating sex hormone levels to incident CVD in men have been inconclusive. For example, low serum testosterone levels have been associated with greater progression of subclinical atherosclerosis in 2 previous investigations (13, 14), but other studies have reported no association of testosterone levels with CVD events (15–21). On a parallel note, low DHEA-S levels have been linked to greater CVD risk in some studies (18, 22–24) but not in other studies (13, 20, 25–29). Investigations

relating serum estradiol levels to CVD risk in men have generally found no statistically significant association (15–20). Some previous investigations were limited by modest sample sizes (14, 17, 18, 22, 25, 26); an insufficient number of CVD events (15–18, 22, 23, 26); and, in some instances, a retrospective study design (17–21, 25, 26). In addition, some reports (15, 22, 24) focused on CVD death (they did not evaluate nonfatal CVD events). Thus, a large prospective community-based study relating sex hormones to CVD risk with adequate power to detect modest potential associations is needed. Accordingly, we evaluated the associations of serum levels of sex hormones that were measured at a routine baseline examination with CVD incidence in a prospectively assembled cohort of participants.

See also:

## Print

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## Web-Only

Appendix Table

Conversion of figure and tables into slides

**METHODS****Study Sample**

The Framingham Heart Study, a prospective study of the risk factors for the development of heart disease and stroke, began in 1948 with the recruitment of 5209 men and women between 30 and 60 years of age who resided in Framingham, Massachusetts (30). The Framingham Offspring Study began in 1971 with the recruitment of 5124 participants who were the children of the original cohort and the spouses of the children (31). Participants in the original cohort are examined every 2 years, while offspring cohort members are assessed every 4 years. At each Framingham Heart Study examination, attendees undergo a physical examination and laboratory assessment of risk factors (31).

We evaluated 2789 men who attended the 17th biennial examination (1981–1983) of the original cohort or the fourth quadrennial examination (1987–1991) of the offspring cohort. We measured serum total testosterone and estradiol levels at these examinations, and we measured DHEA-S levels at the 18th examination (1983–1985) for the original cohort and at the fourth examination for the offspring cohort. We excluded 705 men because of prevalent CVD ( $n = 541$ ) and nonavailable testosterone data ( $n = 164$ ). After exclusions, 2084 men (74.7%; 525 original cohort participants) without previous CVD were eligible. Data on serum estradiol levels and serum DHEA-S levels were available in 2047 men and 1928 participants, respectively. All participants gave written informed consent, and the institutional review board at the Boston Medical Center approved the study protocol.

**Biochemical Assessment of Sex Hormones**

As described previously (32), we measured total testosterone, total estradiol, and DHEA-S levels from serum samples by using radioimmunoassays (Diagnostic Products Corp., Los Angeles, California) for total testosterone (interassay coefficient of variation, 11%), total estradiol (interassay coefficient of variation, 4%), and DHEA-S (interassay coefficient of variation, 11%). We also measured serum luteinizing hormone levels at the baseline examinations (interassay coefficient of variation, 6%).

**Cardiovascular Outcomes**

All participants were under continuous surveillance for the occurrence of CVD events and death. Participants are evaluated periodically at the Framingham Heart Study and through health history updates between examinations (obtained via telephone interviews). Three experienced investigators obtained and reviewed hospitalization and physician office visit records.

We defined incident CVD as coronary heart disease (recognized or unrecognized myocardial infarction, angina pectoris, coronary insufficiency, or coronary heart disease death), cerebrovascular disease (stroke or transient ischemic attack), congestive heart failure (by Framingham criteria), or peripheral vascular disease (intermittent claudication).

**Context**

Studies of the role of endogenous sex hormones in cardiovascular disease (CVD) in men have been inconclusive.

**Contribution**

A total of 2084 men from 2 Framingham Heart Study cohorts had levels of total serum estrogen, testosterone, and dehydroepiandrosterone sulfate (DHEA-S) measured in 1981 to 1985 for the original cohort and in 1987 to 1991 for the offspring cohort. Testosterone and DHEA-S levels were not associated with CVD risk. Estrogen levels were inversely related: Risk for CVD in the highest quartile was 0.68 (95% CI, 0.50 to 0.92) times that in the lowest level.

**Cautions**

Investigators studied only white men and did not study free unbound hormone levels.

**Implications**

Endogenous estrogen may be vasculoprotective in men, which is in contrast to the effects of exogenous estrogen.

—The Editors

Criteria for the diagnoses of cardiovascular events have been described elsewhere (33). We considered that an unrecognized myocardial infarction occurred if we found electrocardiographic evidence of clinically significant loss of R waves or appearance of pathologic Q waves on serial tracings in the absence of a clinically recognized event (33).

We defined the follow-up period a priori as 10 years from the baseline examination (up to 1995 for the original cohort and 2002 for the offspring cohort) to permit equal durations of follow-up of original and offspring cohort participants and because sex hormone levels change considerably with age (34). The analyses that combined the original and offspring cohorts had greater statistical power and allowed us to evaluate participants over a wider age range.

**Statistical Analyses**

Serum testosterone levels were normally distributed. Serum estradiol and DHEA-S levels were skewed and were log-transformed. We examined the associations between baseline sex hormone levels and CVD incidence during follow-up (separate analyses for each hormone). We chose incident CVD as the outcome because the effects of sex hormones are not limited to a given vascular territory and this maximized our statistical power and limited multiple statistical testing (as opposed to analysis for each CVD component). We stratified all analyses by the cohort (offspring cohort vs. original cohort). We verified that the assumption of proportionality of hazards was satisfied for each hormone. We calculated age- and multivariable-adjusted 10-year incidence rates for CVD for each hormone quartile (35).

We prespecified 2 types of models: Primary analyses

Table 1. Baseline Characteristics\*

Characteristic	All Participants	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P Value†
<b>Testosterone (n = 2084)</b>						
Testosterone level						
nmol/L	18.1 (5.8)	11.5 (2.2)	15.8 (1.0)	19.4 (1.1)	25.7 (4.0)	
ng/mL	5.2 (1.7)	3.3 (0.6)	4.5 (0.3)	5.6 (0.3)	7.4 (1.1)	
Participants, n	2084	529	513	512	530	
Age, y	56 (12)	58 (12)	56 (12)	56 (13)	54 (12)	<0.001
Systolic blood pressure, mm Hg	132 (18)	134 (17)	132 (17)	132 (19)	130 (18)	0.23
Diastolic blood pressure, mm Hg	81 (10)	82 (10)	81 (9)	81 (10)	80 (10)	0.001
Antihypertensive medication, %‡	22	26	21	22	18	0.173
Total cholesterol–HDL cholesterol ratio	5.04 (1.58)	5.36 (1.78)	5.06 (1.56)	4.89 (1.44)	4.85 (1.48)	<0.001
Lipid-lowering medication, %‡	3	4	3	2	2	0.008
Diabetes, %	8	11	8	6	7	0.027
Diabetes treatment, %‡	4	5	4	3	3	0.24
BMI, kg/m <sup>2</sup>	27.4 (3.8)	28.3 (4.2)	27.6 (3.9)	27.1 (3.5)	26.6 (3.5)	<0.001
Smokers, %	22	17	18	21	31	<0.001
<b>DHEA-S (n = 1928)</b>						
DHEA-S level, μmol/L	0.64 (0.50)	0.19 (0.07)	0.39 (0.05)	0.62 (0.10)	1.35 (0.48)	
Participants, n	1928	480	484	481	483	
Age, y	55 (12)	63 (11)	57 (10)	52 (10)	47 (10)	<0.001
Systolic blood pressure, mm Hg	131 (18)	136 (18)	131 (18)	129 (17)	129 (17)	<0.001
Diastolic blood pressure, mm Hg	81 (10)	80 (10)	80 (9)	81 (9)	82 (10)	<0.001
Antihypertensive medication, %‡	20	26	22	19	15	0.037
Total cholesterol–HDL cholesterol ratio	5.04 (1.58)	5.24 (1.56)	5.00 (1.55)	4.96 (1.55)	4.97 (1.63)	0.33
Lipid-lowering medication, %‡	3	2	3	3	3	0.047
Diabetes, %	8	11	9	7	4	0.20
Diabetes treatment, %‡	4	7	3	4	1	0.38
BMI, kg/m <sup>2</sup>	27.5 (3.8)	27.7 (4.1)	27.3 (3.5)	27.5 (3.8)	27.6 (3.9)	0.28
Smokers, %	22	16	19	23	32	<0.001
<b>Estradiol (n = 2047)</b>						
Estradiol level						
pmol/L	111.2 (48.8)	55.1 (14.5)	90.4 (9.3)	124.3 (10.0)	174.8 (36.0)	
pg/mL	30.3 (13.3)	15.0 (3.9)	24.6 (2.5)	33.8 (2.7)	47.6 (9.8)	
Participants, n	2047	512	510	511	514	
Age, y	56 (12)	55 (12)	57 (12)	56 (12)	56 (13)	0.21
Systolic blood pressure, mm Hg	132 (18)	131 (17)	130 (17)	133 (19)	134 (18)	0.011
Diastolic blood pressure, mm Hg	81 (10)	81 (10)	80 (9)	81 (10)	81 (10)	0.23
Antihypertensive medication, %‡	22	17	19	25	27	<0.001
Total cholesterol–HDL cholesterol ratio	5.03 (1.56)	5.14 (1.62)	5.11 (1.61)	4.91 (1.51)	4.96 (1.50)	0.012
Lipid-lowering medication, %‡	3	3	2	1	4	0.69
Diabetes, %	8	7	8	7	10	0.21
Diabetes treatment, %‡	4	2	5	3	6	0.071
BMI, kg/m <sup>2</sup>	27.4 (3.8)	27.4 (3.6)	27.4 (4.1)	27.3 (3.9)	27.6 (3.8)	0.36
Smokers, %	22	20	21	21	25	0.076

\* Values are means (SDs), unless otherwise indicated. Quartile 1 is the lowest quartile, and quartile 4 is the highest quartile. BMI = body mass index; DHEA-S = dehydroepiandrosterone sulfate; HDL = high-density lipoprotein.

† P value for trend among quartiles from an age-adjusted model.

‡ Indicates proportion of whole sample receiving treatment (not just individuals with condition).

evaluated the sex hormones as continuous variables to maximize statistical power (model A), and additional analyses compared CVD risk in hormone quartiles 2 to 4 with that in quartile 1 (referent) (model B). These models facilitated assessment of potential nonlinear relations.

For each hormone, we used proportional hazards regression (36) that adjusted for 1) age alone and 2) age, smoking, systolic and diastolic blood pressure, antihypertensive medication use, ratio of total and high-density lipoprotein (HDL) cholesterol, diabetes mellitus, and body mass index (BMI). We evaluated models adjusted for age alone because some risk factors may fall along the causal pathway from estradiol to CVD. Adjustment for BMI was performed because adiposity is a strong correlate of sex

hormone levels (37) and of sex hormone–binding globulin (38). Sex hormone–binding globulin strongly influences measurements of total circulating sex hormone levels and the amount of bound hormone versus free hormone. We also evaluated models without BMI as a covariate to assess whether adjustment for BMI attenuated relations of sex hormones with CVD and models that were adjusted additionally for the use of aspirin or lipid-lowering medications, alcohol consumption, and education level. We incorporated statistical interaction terms to evaluate effect modification by age, BMI, smoking status, systolic blood pressure, and total cholesterol–HDL cholesterol ratio.

To gain additional insights into potential nonlinearity of associations between hormone levels and CVD risk, we

examined generalized additive Cox models using penalized splines (39, 40) for hormones that were statistically significantly related to CVD risk. Because potential relationships of sex hormones to CVD risk may be mediated by the development of risk factors during follow-up, we prespecified examination of time-dependent Cox models (updating established CVD risk factors [covariates in the multivariable-adjusted proportional hazards regression] every 4 years at follow-up examinations) for analyses of any sex hormone that was statistically significantly related to CVD events in the initial analyses.

### Additional Analyses

We performed secondary analyses relating CVD risk to the estradiol–testosterone ratio (37, 41). In addition, we investigated whether hypogonadism was related to CVD risk. We defined hypogonadism empirically as a serum testosterone level less than 10.4 nmol/L (<3 ng/mL) or a luteinizing hormone level of 20 IU/L or greater, which is consistent with a previous report from the Framingham Heart Study (32).

We performed all analyses by using the PHREG procedure in SAS software (SAS Institute Inc., Cary, North Carolina). We generated the display of the multivariable-adjusted hazard ratio on a logarithmic scale against the hormone levels by using S-PLUS, version 7.0.0 (Insightful Corp., Seattle, Washington). We considered a 2-sided *P* value less than 0.050 to be statistically significant.

### Role of the Funding Sources

Funding for the study was provided by a Bergmarks travel grant, a Viking Björks Hedersledamotstipendium, a Capio travel grant, and the Thuréus Foundation and through research grants from the National Heart, Lung, and Blood Institute. The funding sources did not play any

role in the design, conduct, or analysis of the study or in the decision to submit the manuscript for publication.

## RESULTS

### Baseline Characteristics

Table 1 displays the baseline characteristics of our sample according to the quartile of sex hormones. Age, diastolic blood pressure, BMI, total cholesterol–HDL cholesterol ratio, and diabetes prevalence decreased across serum testosterone quartiles, whereas the proportion of smokers increased. Age, systolic blood pressure, and antihypertensive medication use decreased across serum DHEA-S quartiles, while diastolic blood pressure and the proportion of smokers increased. Total cholesterol–HDL cholesterol ratio decreased across serum estradiol quartiles, while systolic blood pressure and antihypertensive medication use increased. Serum log estradiol was correlated weakly with both testosterone (Pearson  $r = 0.12$ ;  $P < 0.001$ ) and log DHEA-S ( $r = 0.16$ ;  $P < 0.001$ ). Serum testosterone was correlated weakly with log DHEA-S ( $r = 0.08$ ;  $P < 0.001$ ).

### Incidence of CVD Events

Over a 10-year follow-up, 386 men (18.5%; 181 original cohort participants and 205 offspring participants) experienced a first CVD event (data for the largest sample, that is, individuals with available data on serum testosterone levels: coronary heart disease, 219 participants [56.7%]; cerebrovascular disease, 77 participants [20%]; congestive heart failure, 39 participants [10.1%]; peripheral vascular disease, 44 participants [11.4%]; and other CVD, 7 participants [1.8%]). Age-adjusted CVD incidence rates decreased linearly across quartiles of serum testosterone (Ta-

Table 2. Cardiovascular Disease Event Rates according to Quartiles of the Sex Hormones\*

Quartile	Events/Participants at Risk, n/n	Follow-up, person-years	Age-Adjusted 10-Year CVD Incidence (95% CI), %	Multivariable-Adjusted 10-Year CVD Incidence (95% CI), %
<b>Testosterone (n = 2084)</b>				
Quartile 1	110/529	4427	21.96 (17.98–25.62)	20.22 (14.32–25.29)
Quartile 2	107/513	4325	21.54 (17.61–25.18)	21.26 (15.09–26.51)
Quartile 3	91/512	4355	19.64 (15.72–23.26)	20.38 (14.27–25.58)
Quartile 4	78/530	4555	18.48 (14.51–22.14)	18.55 (12.66–23.59)
<b>DHEA-S (n = 1928)</b>				
Quartile 1	137/480	3710	20.86 (17.07–24.39)	19.97 (14.01–25.06)
Quartile 2	87/484	4077	18.88 (14.92–22.52)	18.89 (12.89–23.96)
Quartile 3	67/481	4334	18.96 (14.48–23.01)	18.97 (12.61–24.29)
Quartile 4	56/483	4319	20.07 (14.86–24.67)	19.57 (12.65–25.25)
<b>Estradiol (n = 2047)</b>				
Quartile 1	94/512	4365	21.50 (17.34–25.32)	21.54 (15.16–26.94)
Quartile 2	107/510	4244	23.04 (18.86–26.87)	22.52 (16.04–27.98)
Quartile 3	97/511	4364	21.09 (17.04–24.82)	21.69 (15.29–27.09)
Quartile 4	83/514	4362	16.97 (13.38–20.32)	15.62 (10.57–20.06)

\* Multivariable-adjusted rates adjusted for age, smoking, systolic and diastolic blood pressure, hypertension treatment, total cholesterol–high-density lipoprotein cholesterol ratio, diabetes mellitus, and body mass index. CVD = cardiovascular disease; DHEA-S = dehydroepiandrosterone sulfate.

**Table 3. Relations of Endogenous Sex Hormones and Cardiovascular Disease Incidence: Age- and Multivariable-Adjusted Cox Regression\***

Variable	Age-Adjusted Model		Multivariable-Adjusted Model	
	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
<b>Testosterone (n = 2084)</b>				
Model A: as a continuous variable				
1-SD increase in testosterone	0.95 (0.85–1.05)	0.31	0.99 (0.88–1.10)	0.83
Model B: multcategory models				
Quartile 1	1.0 (referent)		1.0 (referent)	
Quartile 2	1.01 (0.77–1.31)	0.96	1.09 (0.83–1.44)	0.53
Quartile 3	0.89 (0.67–1.18)	0.42	1.03 (0.77–1.37)	0.83
Quartile 4	0.83 (0.62–1.12)	0.22	0.92 (0.68–1.25)	0.61
<b>DHEA-S (n = 1928)</b>				
Model A: as a continuous variable				
1-SD increase in log DHEA-S	0.98 (0.87–1.11)	0.73	1.02 (0.90–1.15)	0.72
Model B: multcategory models				
Quartile 1	1.0 (referent)		1.0 (referent)	
Quartile 2	0.91 (0.69–1.20)	0.51	0.96 (0.72–1.28)	0.77
Quartile 3	0.87 (0.63–1.19)	0.38	0.91 (0.66–1.25)	0.56
Quartile 4	0.98 (0.69–1.39)	0.89	1.01 (0.71–1.45)	0.95
<b>Estradiol (n = 2047)</b>				
Model A: as a continuous variable				
1-SD increase in log estradiol	0.92 (0.84–1.01)	0.09	0.90 (0.82–0.99)	0.035
Model B: multcategory models				
Quartile 1	1.0 (referent)		1.0 (referent)	
Quartile 2	1.06 (0.80–1.40)	0.68	1.03 (0.77–1.36)	0.85
Quartile 3	0.94 (0.70–1.24)	0.65	0.95 (0.71–1.27)	0.71
Quartile 4	0.75 (0.56–1.02)	0.06	0.67 (0.49–0.91)	0.010

\* Multivariable-adjusted models adjusted for age, smoking, systolic and diastolic blood pressure, hypertension treatment, total cholesterol–high-density lipoprotein cholesterol ratio, diabetes mellitus, and body mass index. DHEA-S = dehydroepiandrosterone sulfate.

ble 2). However, evaluation of multivariable-adjusted rates demonstrated lower rates only for the highest testosterone quartile relative to the other quartiles. For DHEA-S, we observed no consistent pattern across quartiles. For estradiol, the highest quartile was associated with lower age- and multivariable-adjusted CVD incidence rates relative to the lower 3 quartiles (Table 2).

### Testosterone Levels, DHEA-S Levels, and CVD Risk

Serum levels of testosterone or DHEA-S were not associated with CVD risk in either age-adjusted or multivariable-adjusted models (Table 3). We observed no effect modification by age, BMI, smoking, systolic blood pressure, and total cholesterol–HDL cholesterol ratio. On the basis of 95% CIs of the hazard ratios for testosterone and DHEA-S, we cannot exclude effects smaller than about a 10% increase or decrease in the CVD hazard associated with a 1-SD increase in either hormone.

### Estrogen and CVD Risk

An SD increase in log estradiol was statistically significantly associated with an 8% to 10% lower CVD risk (Table 3). Men in the highest quartile of estradiol had a 33% multivariable-adjusted lower risk for CVD events compared with men in the first quartile. These results remained unchanged after additional adjustment for aspirin use, lipid-lowering medication use, alcohol consumption, and education. Also, models with and without BMI

yielded identical results. Examination of multivariable-adjusted regression splines demonstrated a decrease in CVD hazard with increasing estradiol levels (Figure). The association of estradiol levels with lower CVD risk remained robust in time-dependent models, updating covariates at follow-up examinations (hazard ratio, 0.90 [95% CI, 0.82 to 0.99] per SD increment in log estradiol [ $P = 0.028$ ] and 0.68 [CI, 0.50 to 0.92] for quartile 4 vs. quartile 1 [ $P = 0.012$ ]).

We observed a statistically significant interaction ( $P = 0.040$ ) with age for serum estradiol but not for other covariates. Therefore, we repeated analyses, stratifying by age ( $\leq 56$  years vs.  $>56$  years [median age of sample]). Log estradiol was associated with CVD incidence in older (age  $> 56$  years) participants (hazard ratio, 0.86 [CI, 0.78 to 0.96] per SD increase [ $P = 0.005$ ] and 0.56 [CI, 0.39 to 0.81] for quartile 4 vs. quartile 1 [ $P = 0.002$ ]) but not in younger (age  $\leq 56$  years) people (hazard ratio, 1.11 [CI, 0.89 to 1.38] per SD increment [ $P = 0.36$ ] and 1.07 [CI, 0.60 to 1.90] for quartile 4 vs. quartile 1 [ $P = 0.82$ ]). We obtained similar results in time-dependent models, updating covariates at follow-up examinations.

### Additional Analyses

To evaluate the sensitivity of our results to extreme values of estradiol, we repeated analyses in a subset of 2025 individuals after excluding 22 participants with estradiol

values greater than 245.9 pmol/L (>67 pg/mL) (cut-point for quartile 3 plus 1.5 times the interquartile range [42]). The association of higher estradiol levels with lower CVD risk remained robust in these analyses, and the effect modification by age remained (data not shown).

In supplementary analyses evaluating the estradiol–testosterone ratio, a higher ratio was associated with a lower CVD risk (Appendix Table, available at [www.annals.org](http://www.annals.org)). Models incorporating the estradiol–testosterone ratio did not improve discrimination ability compared with those with estradiol alone (c-statistic for both models, 0.76 [CI, 0.73 to 0.78]).

In additional analyses, we categorized 140 men as having hypogonadism. Thirty of 140 men (21.4%) with hypogonadism had a CVD event on follow-up compared with 347 of 1883 men (18.4%) without hypogonadism. We did not detect a statistically significant relationship between hypogonadism and CVD risk (multivariable-adjusted hazard ratio, 0.83 [CI, 0.57 to 1.22];  $P = 0.34$ ).

## DISCUSSION

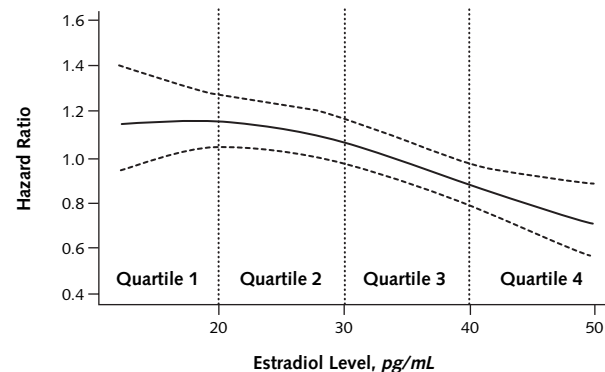
### Principal Findings

In our large community-based sample of men, we observed that a higher serum estradiol level was associated with a lower risk for CVD events after adjustment for established cardiovascular risk factors. We observed effect modification by age, with an inverse relationship of estradiol and CVD risk observed in older men but not in younger men. We did not observe any association between serum testosterone level or DHEA-S level and CVD incidence, although our investigation cannot exclude modest cardioprotective effects of either hormone.

### Comparisons with the Literature

Several previous studies have investigated the relationship between serum estradiol levels and CVD in men (15–20). Most of these studies were limited by small sample sizes and a case–control design (17–20). Two previous prospective community-based studies reported a lack of association of serum estradiol levels and CVD risk (15, 16). Differences in the size and age distribution of study samples, the follow-up durations, and the choices of outcome events evaluated may partly explain why the results of these studies differed from those of our investigation. The report based on the Rancho Bernardo Study (15) had a smaller sample size ( $n = 1009$ ), but its age distribution (age 40 to 79 years) and follow-up duration (12 years) were similar to those of our investigation. However, the investigators evaluated only cardiovascular death and consequently accrued fewer outcome events (88 fatal CVD events). Nonetheless, the age-adjusted event rates in that study were 12% lower in the highest tertile of estradiol relative to the lowest tertile (9.1% vs. 10.3%), although the difference was not statistically significant. The report from the Caerphilly Prospective Study (16) was based on a slightly larger sample ( $n = 2512$ ) than ours, but the par-

**Figure. Multivariable-adjusted association of serum levels of estradiol and cardiovascular disease risk.**



The solid line shows the hazard ratio estimated as a function of penalized regression splines of estradiol to the baseline hazard. Dashed lines are 95% confidence limits for the hazard ratio. Dotted lines represent quartile limits of serum estradiol levels. Hazard ratios greater than 1.0 indicate increased hazard compared with the baseline hazard, and hazard ratios less than 1.0 indicate decreased hazard compared with the baseline hazard. To convert estradiol levels from pg/mL to pmol/L, multiply value by 3.671.

ticipants were younger (age range, 49 to 59 years) and the duration of follow-up was shorter (5 years). Furthermore, the investigators focused only on coronary heart disease outcomes (153 events). In that study, the odds ratio for coronary heart disease per SD increment in estradiol was 1.11 (CI, 0.92 to 1.34), suggesting that protective effects of estradiol smaller than 8% or harmful effects of estradiol smaller than 34% could not be excluded. Additional factors that distinguish our study from these previous reports include the continuous surveillance of our participants for CVD events and the specific examination of effect modification by age.

Of note, we observed an association of higher estradiol levels with lower CVD risk in older men but not in younger men. The age-associated increase in vascular risk paralleled by decreasing sex hormone levels may account for the greater propensity for CVD among older individuals with lower estradiol levels. Investigators relating estrogen receptor polymorphisms to CVD risk have reported similar effect modification by age: An association was observed in older but not in younger individuals (43).

Our finding that serum levels of testosterone did not predict future cardiovascular events is in accordance with previous longitudinal reports (15–21). However, our findings differ from those of cross-sectional reports (reviewed elsewhere [5]) that have noted lower testosterone levels in patients with prevalent CVD. Other recent studies have noted an association between longitudinal testosterone levels and arterial stiffness (44) and progression of subclinical atherosclerosis (13, 14). The reasons for the discrepancy between studies of progression of subclinical atherosclerosis and prospective studies on CVD incidence (including our

study) are unclear and merit further investigation. The lack of association of DHEA-S levels with CVD events in our sample should add to previous evidence (13, 20, 25–29), negating a primary role for this hormone in the pathogenesis of CVD in men.

### Possible Mechanisms Linking Estradiol Levels to CVD Risk

The biological effects of estrogen are less well defined in men than in women, and whether estrogen has sex-specific effects is not clear. In men, estrogen is mainly synthesized by local tissue aromatization of androgenic precursors from the testes and adrenal gland, and estrogen levels do not seem to decrease with age to the same extent as testosterone or DHEA-S levels (45).

Estrogen may lower CVD risk in men through several mechanisms. First, estrogen may have beneficial effects on CVD risk factors, including blood pressure, lipid levels, and glycemia (46–49). We accounted for this possibility by adjusting for traditional risk factors at baseline and as time-varying covariates. The observation that estrogen remained inversely associated with CVD incidence even after adjustment for standard risk factors suggests that favorable influences on risk factors may not be the sole mechanism for our findings. Second, estrogen favorably influences nontraditional CVD risk factors, such as homocysteine levels, hemostatic factors, inflammation, and endothelial function (7, 48, 50–52), that may translate into a lower CVD risk. Third, higher estradiol levels may reduce susceptibility to ischemia-induced damage in target organs. Experimental studies have shown that estrogen administration protects against ischemia–reperfusion injury in both the brain (53) and the heart (54).

### Strengths and Limitations

The community-based sample, continuous surveillance for CVD, adjudication of events blinded to hormone levels, and ability to adjust for several CVD risk factors (at baseline and during follow-up) strengthen our investigation. However, several limitations of our study merit comment. First, we measured sex hormones at a single baseline examination. Levels of sex hormones decrease with increasing age in men (55). However, single measures of sex hormone levels provide a reasonable estimate of long-term levels (56). Second, we measured total testosterone and estradiol levels, not those of the unbound hormones. Free hormones are more biologically significant (57). Total sex hormone measurements are substantially influenced by the levels of their binding proteins. Since adiposity is a key correlate of sex hormone–binding globulin, we adjusted for BMI in our analyses. Nonetheless, additional prospective community-based studies are warranted to relate free sex hormone levels to CVD risk. Third, our sample consisted of white men, which limits the generalizability of our findings to other races or ethnicities and to women. Fourth, we performed several statistical tests by relating 3 sex hormones to CVD risk, although we defined all anal-

yses a priori. The consistency of results for estradiol levels across different models makes it unlikely that the observed association was due to multiple testing. Fifth, in our middle-aged sample, few men had very low testosterone levels or hypogonadism, which may preclude examination of effects of major testosterone deficiency. Finally, the relationship between serum testosterone levels and CVD may vary according to genetically determined differences in androgen sensitivity, a premise that we did not evaluate.

### Implications

Nearly 40 years ago, 2 large randomized clinical trials of oral estrogen observed an increased risk for CVD in men treated with exogenous estrogen (58, 59). Since then, accumulating evidence underscores the potential cardioprotective effects of endogenous estrogens (2, 4, 6, 7, 52, 60). Our observational data emphasize the importance of distinguishing influences of endogenous estrogens from the effects of exogenous estrogen supplementation. If our findings are replicated in other studies, the pathophysiologic mechanism by which higher endogenous estrogen reduces CVD incidence in men is important to investigate. However, only clinical trials can determine whether treatment with estrogen-modulating agents can reduce CVD incidence in high-risk men with low serum estrogen levels.

### Conclusions

In our community-based sample, higher serum estradiol levels were associated with a lower risk for CVD events in older men. Our findings are consistent with the hypothesis that endogenous estrogen has vasculoprotective influences in men.

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**Appendix Table. Estrogen–Testosterone Ratio and Cardiovascular Disease Risk\***

Variable	Age-Adjusted Model		Multivariable-Adjusted Model	
	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
<b>Model A: as a continuous variable</b>				
1-SD increase in estrogen–testosterone ratio	0.94 (0.85–1.04)	0.23	0.89 (0.81–0.99)	0.035
<b>Model B: multcategory models</b>				
Quartile 1	1.0 (referent)		1.0 (referent)	
Quartile 2	1.19 (0.90–1.59)	0.22	1.10 (0.82–1.48)	0.51
Quartile 3	0.90 (0.67–1.22)	0.49	0.87 (0.64–1.17)	0.35
Quartile 4	0.92 (0.68–1.23)	0.56	0.79 (0.59–1.07)	0.130

\* Estrogen–testosterone ratio is estrogen values (in pg/mL) divided by testosterone values (in ng/mL). Multivariable-adjusted models are adjusted for age, smoking, systolic and diastolic blood pressure, hypertension treatment, total cholesterol–high-density lipoprotein cholesterol ratio, diabetes mellitus, and body mass index.