Association of Sex Hormones, Aging, and Atrial Fibrillation in Men
The Framingham Heart Study

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Background—Endogenous sex hormones have been related to cardiovascular outcomes and mortality. We hypothesized that sex hormones are related to atrial fibrillation (AF) in a community-based cohort of middle-aged to older men.

Methods and Results—We examined testosterone, estradiol, and dehydroepiandrosterone sulfate in relation to incident AF in men participating in the Framingham Heart Study. We assessed the 10-year risk of AF in multivariable-adjusted hazard models. The cohort consisted of 1251 men (age, 68.0±8.2 years), of whom 275 developed incident AF. We identified a significant interaction between age and testosterone and, therefore, stratified men into age 55 to 69 years (n=786), 70 to 79 years (n=351), and ≥80 years (n=114). In men aged 55 to 69 years, each 1 SD decrease in testosterone was associated with hazard ratio (HR) 1.30 (95% confidence interval [CI], 1.07–1.59) for incident AF. The association between testosterone and 10-year incident AF in men 70 to 79 years did not reach statistical significance. In men ≥80 years, a 1 SD decrease in testosterone was associated with HR 3.53 (95% CI, 1.96–6.37) for AF risk. Estradiol was associated with incident AF (HR, 1.12; 95% CI, 1.01–1.26). Dehydroepiandrosterone sulfate had a borderline association with risk of AF that was not statistically significant (HR, 1.12; 95% CI, 0.99–1.28).

Conclusions—Testosterone and estradiol are associated with incident AF in a cohort of older men. Testosterone deficiency in men ≥80 years is strongly associated with AF risk. The clinical and electrophysiological mechanisms underlying the associations between sex hormones and AF in older men merit continued investigation.

Key Words: aging ■ atrial fibrillation ■ epidemiology ■ men

Older adults are at increased risk for atrial fibrillation (AF). The risk of incident AF in men is approximately twice that of women.1 As a result, we consider identifying sex-specific risk factors as relevant to the epidemiology of AF. Furthermore, AF risk increases 2-fold for each progressive decade of aging,2 such that AF has been identified as a disease principally of older age. Endogenous sex hormones (testosterone, estrogen, and dehydroepiandrosterone sulfate [DHEA-S]) are associated with cardiovascular disease and mortality outcomes in older men.3 We considered the prospective association between endogenous sex hormones and AF risk in men. We hypothesized that decreased levels of testosterone, estradiol, and DHEA-S would be associated with increased AF risk. Second, because sex steroid hormones vary with aging, we sought to examine whether their association with AF risk varied by age.

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with AF (n=74).15 All participants provided written, informed consent at each examination, and the Boston University Medical Center Institutional Review Board approved the study protocols.

Sex Hormone Measurements
Sex hormones included serum total testosterone, estradiol, and DHEA-S levels (Diagnostic Products Corporation, Los Angeles, CA) as described previously.11 Hormone radioimmunoassays had reliable interassay coefficients of variation (11% for total testosterone, 4% for total estradiol, 11% for DHEA-S).

Risk Factor Assessments
Framingham Heart Study examinations included a standardized physician examination, medical interview, and interim history. Risk factors in the present analysis were selected because of their established associations with AF in the Framingham Heart Study and other community-based cohorts. Body mass index (kg/m²) was determined as the ratio of weight to height squared. Systolic blood pressure was obtained by a physician during the Framingham examination and determined as the mean of 2 seated measurements taken in a 5-minute interval. Self-report of medications was used to establish hypertension treatment, PR interval, significant murmur, and prevalent heart failure.

Determination of AF
The Framingham Heart Study has conducted prospective surveillance of participants for ascertainment of cardiovascular outcomes. AF was ascertained from the ECG, Holter, telemetry, or other rhythm monitoring at the Framingham examination or evaluation elsewhere and was adjudicated by 2 Framingham Heart Study cardiologists. Mortality data were ascertained from death certificates, hospital or institutional records, obituaries, or direct notification. We followed participants for up to 10 years from the examination at which the hormone levels were measured or up to death during the 10-year follow-up period.

Statistical Analyses
We examined the distributions of continuous variables using both numeric (means, SDs, medians, and interquartile ranges) and graphical summaries. We calculated the frequencies of categorical variables. We assessed the normality of hormone measurements; we determined that total testosterone levels were distributed normally and natural log-transformed DHEA-S and estradiol to approximate normal distributions. We examined the age-adjusted pairwise Pearson correlations between the 3 hormone measurements and AF risk factors. We assessed the proportionality of hazards between each hormone measurement and AF. We examined the associations between a 1 SD change for each of the hormonal exposures and the 10-year risk of AF using multivariable Cox proportional hazards regression analyses. We dichotomized testosterone at 300 ng/dL, a standard clinical threshold, and examined the risk of 10-year incident AF for testosterone <300 ng/dL using ≥300 ng/dL as the referent. We identified significant interaction by age in the association between testosterone and AF risk; but not the other hormones. We consequently conducted age-stratified analyses of the association of testosterone and AF. We adjusted all Cox models for AF risk factors identified from a validated AF risk prediction algorithm,11 including age, body mass index, systolic blood pressure, hypertension treatment, diabetes mellitus, PR interval, significant murmur, and prevalent heart failure. Next, we used multivariable-adjusted penalized splines to examine the relation of testosterone and 10-year AF risk by age category. We used the median value of testosterone (450 ng/dL) as the referent. Because of the mutual relations between AF and heart failure, we conducted a prespecified secondary analysis to examine if interim heart failure modified the association between the sex hormones and AF. We considered a 2-sided P value <0.05 as statistically significant and performed all analyses using SAS version 9.3 (SAS Institute, Cary, NC).

Results
The total study sample consisted of 1251 participants (age, 68.0±8.2 years). Heart failure was prevalent in only 2% of the cohort. The remaining clinical characteristics and hormone measures are summarized in Table 1. Because of the testosterone–age interaction, we age-stratified the cohort: 55 to 69 years (n=786), 70 to 79 years (n=351), and ≥80 years (n=114). We compared covariates at baseline by AF outcome in Table I in the Data Supplement. All covariates were included in multivariable-adjusted models relating the baseline assessments of hormone levels to incident AF.

Age-adjusted Pearson correlations between clinical covariates and the hormone measures are shown in Table 2. All correlations were of modest magnitude. Testosterone had a moderate correlation with body mass index (r=−0.23; P<0.0001). Sex hormones had moderate but highly significant associations with each other (testosterone and estradiol: r=0.30; P<0.0001; estradiol and DHEA-S: r=0.28; P<0.0001).

During 10-year follow-up, we observed 275 cases of incident AF. The 10-year hazard ratio (HR) for incident AF in participants with testosterone levels <300 ng/dL was 1.28 (95% confidence interval [CI], 0.90–1.28). We observed a statistical interaction between age and testosterone (P=0.001) in the multivariable-adjusted model including smoking, body mass index, systolic blood pressure, hypertension treatment, diabetes mellitus, PR interval, significant murmur, and prevalent heart failure. We stratified by age category to determine the association between testosterone and 10-year risk of incident AF.

In men 55 to 69 years (n=786), there were 133 (16.9% of 786) AF events. Each 1 SD testosterone decrease was associated with 1.28 (95% CI, 0.90–1.28) for incident AF. The 10-year hazard ratio (HR) for incident AF in participants with testosterone levels <300 ng/dL was 1.28 (95% CI, 1.07–1.59) for incident AF after multivariable adjustment (Table 3). In men 70 to 79 years (n=351), there were 116 (33.1% of 351) AF events. We observed a relation between testosterone and risk of incident AF that was not statistically

| Table 1. Baseline Participant Characteristics and Measures of Endogenous Sex Hormones |
|---------------------------------|-----------------|-----------------|
| Participants (n=1251)           | Clinical characteristics |
|                                 | Age, y           | 68.0±8.2        |
|                                 | Smoking, present or past | 263 (21%) |
|                                 | Body mass index, kg/m² | 26.7±3.8        |
|                                 | Systolic blood pressure, mm Hg | 138±19       |
|                                 | Hypertension treatment | 436 (35%)      |
|                                 | Diabetes mellitus | 137 (11%)       |
|                                 | PR interval duration, ms | 174±28         |
|                                 | Significant murmur* | 61 (5%)         |
|                                 | Heart failure      | 23 (2%)         |
|                                 | Hormone levels     |                 |
|                                 | Testosterone, ng/dL | 450 (360, 560)  |
|                                 | Estradiol, pg/mL   | 23.5 (15.2, 32.9) |
|                                 | DHEA-S, μg/dL      | 112.0 (67.9, 173.0) |

Continuous variables presented as mean±SD and categorical data as frequency (percentage). Estradiol and dehydroepiandrosterone sulfate (DHEA-S) are depicted as median 25th percentile and 75th percentile because of their nonnormal distribution.

*As defined by text.
significant (HR, 1.14; 95% CI, 0.91–1.44). In men ≥80 years (n=114), there were 26 (22.8% of 114) AF events, where every 1 SD decrease in testosterone was associated with HR 3.53 (95% CI, 1.96–6.37) for AF risk. Multivariable-adjusted regression splines (Figure 1) show the varied relation of testosterone to AF hazard by age strata. Cohort participants in the highest age group (≥80 years) have a nonlinear relation between decreased testosterone and markedly elevated AF risk. In contrast, the other 2 age groups demonstrate a more linear relation with decreasing risk of AF across increasing testosterone.

No statistical interaction was observed between age and estradiol or DHEA-S. A 1 SD decrease in natural log-transformed estradiol was associated with HR 1.12 (95% CI, 1.01–1.26) for the 10-year risk of AF. The 1 SD decrease in natural log-transformed DHEA-S and AF did not reach statistical significance (HR, 1.12 per 1 SD decrease in DHEA-S; 95% CI, 0.99–1.28).

In secondary analysis, the multivariable estimates were not substantively altered by adjusting for interim heart failure during the course of 10-year follow-up (Table II in the Data Supplement).

### Discussion

We examined the association between endogenous sex hormones and AF in middle-aged to older men. We observed a significant interaction for age and testosterone and performed an age-stratified analysis that identified an association between decreased testosterone and AF risk estimates. Men 55 to 69 years had a 1.3-fold and men ≥80 years had a 3.5-fold increased risk of AF with every 1 SD decrease in testosterone. Both hypogonadism (ie, testicular senescence) and AF are common and contribute significant morbidity in older men. Identifying their relation has potential implications for the prevention of AF in aging men.

To our knowledge, studies of sex hormones in men and their relation to AF are limited. A single-center study found that men with AF had decreased measures of endogenous testosterone compared with matched controls.15 In comparison to our study, the cohort was younger, smaller (n=58), institutional-based, and cross-sectional. However, testosterone levels in both AF and matched cohorts were similar to those quantified in our study. A case series additionally described treatment of 2 men with AF with exogenous testosterone for rhythm control.14 Findings have not been validated in observational or clinical trial cohorts.

In our analysis with men 55 to 69 and ≥80 years, the association between decreased testosterone and AF reached statistical significance. Although there was a trend toward the association between decreased testosterone and AF in men 70 to 79 years, the association did not achieve statistical significance (HR, 1.14; 95% CI, 0.91–1.44). There are several possible reasons for the finding. Men in the intermediate age range (70–79 years) may have competing morbidity, that is, risk of death, which eliminates the risk of AF. The increasing number of comorbidities with aging included in the multivariable adjustment may attenuate the association between reduced testosterone and AF. Likewise, it is possible that men ≥80 years, having survived longer, are, therefore, a healthier cohort, such that the relation of hypogonadism and AF becomes more pronounced.

The literature describing estradiol and cardiovascular risk in men has been inconsistent. Elevated estradiol levels were associated with a 2-fold increased risk of stroke in older men in the Honolulu-Asia Aging Study.15 In contrast, a meta-analysis examining estradiol and cardiovascular risk did not report a consistent protective or adverse effect of the hormone in healthy men.16

### Potential Mechanisms

Multiple clinical and physiological mechanisms may underlie the association of decreased endogenous testosterone and AF in older men. The pathway from reduced testosterone to AF is heterogeneous and multifactorial, as we have sought to demonstrate in Figure 2. Most evident is the relation of testosterone to established risk factors for AF. Cross-sectional studies have related decreased testosterone to higher burden of body fat, dyslipidemia, inflammatory markers, and hypertension.17–21 In community-based studies, obesity and metabolic syndrome are associated with adverse atrial electric remodeling, prospective development of AF;22–24 and inversely related to testosterone in older men.25,26 Diabetes mellitus, hypertension, and smoking constitute additional, established risk factors for AF that also have been associated with reduced endogenous sex hormones in men.17,27,28

Inflammation may further mediate the association between testosterone and AF. B-type natriuretic peptide has been associated with decreased testosterone in men.29 The associations between circulating inflammatory markers and natriuretic peptides with
long-term incident AF are well characterized. The joint effects of inflammation and sex hormones on atrial remodeling and AF remain an area of active investigation. Although previous studies have established the association of multiple and diverse biomarkers with AF, our study is not able to discern whether decreased testosterone potentiates these relations. Similarly, we determined not to assess the contributions of sex hormones to risk prediction using standard reclassification and discrimination metrics. Additional investigation in larger cohorts may assess the relative contributions of sex hormones toward risk models.

Altered atrial electrophysiology may provide an additional mechanism for the association we identified between sex steroid hormones and AF. Animal models have demonstrated that atrial electrophysiology is altered by experimental modification of systemic hormones. Orchiectomized rats demonstrated greater repetitive atrial responses with left atrial pacing compared with controls. The difference in atrial response, associated with increased ryanodine receptor type 2 and sodium–calcium exchange, attenuated with the administration of testosterone. Electrophysiological studies demonstrate age-related changes in atrial electrophysiology and electroanatomy. The relation of endogenous sex hormones and electrophysiology merits continued investigation.

Interim cardiovascular events may also contribute toward the relation of sex hormones and AF in men. Testosterone deficiency, or hypogonadism, is an established risk factor for the progression of atherosclerosis and has been related to atheroma progression and vascular dysfunction. In men with prevalent heart failure, testosterone deficiency has been related to symptom exacerbation, New York Heart Association functional classification, and survival. Up to 37% of men with heart failure have been identified as testosterone-deficient, with the severity of deficiency correlating directly with symptoms and prognosis. In men with heart failure, elevated estradiol is associated with increased mortality risk (HR, 4.17; 95% CI, 2.33–7.45). Heart failure and AF are highly interrelated and may represent another mechanism...
linking endogenous sex hormones and AF. However, it is notable that the observed relations between sex hormones and AF persisted after adjusting for interim heart failure.

Clinical Implications

Our findings have important clinical implications because both hypogonadism and AF are relatively common conditions in older men. Identifying modifiable risk factors has relevance for AF prevention. A recent systematic review of testosterone replacement identified the absence of large, well-designed clinical trials to investigate the effect of repletion on the prevention of cardiovascular events. Whether testosterone repletion has any role in AF prevention has similarly not been demonstrated in a clinical trial. A retrospective analysis identified an association between testosterone repletion and cardiovascular events in a veteran cohort selected for a history of angiography and low testosterone. To our knowledge, the effects of testosterone replacement on AF risk have not yet been investigated. Including AF as an end point in clinical trials investigating testosterone and cardiovascular outcomes has significant importance.

Strengths and Limitations

Our investigation has important strengths. We conducted a longitudinal analysis in a community-based cohort of men spanning mid- to late adulthood. Covariates relevant to the examined outcome were ascertained using a standardized approach. Framingham Heart Study surveillance enhanced the accuracy and outcome were ascertained using a standardized approach. Framing mid- to late adulthood. Covariates relevant to the examined longitudinal analysis in a community-based cohort of men spanning mid- to late adulthood. Covariates relevant to the examined outcome were ascertained using a standardized approach. Framingham Heart Study surveillance enhanced the accuracy and outcome were ascertained using a standardized approach. Framing mid- to late adulthood. Covariates relevant to the examined outcome were ascertained using a standardized approach. Framingham Heart Study surveillance enhanced the accuracy and outcome were ascertained using a standardized approach. Framingham Heart Study surveillance enhanced the accuracy and identification of incident AF and appropriate censoring for mor-

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The study has several limitations as well. Our cohort constituted of middle-aged to older men of European descent; the generalizability to women, younger adults, and other races/ethnicities is unknown. A larger cohort with more events would have superior statistical power. Furthermore, events in the ≥80 age group were few, and the wide CIs around the estimates for this age group indicate statistical imprecision. Additional studies are needed with larger numbers of older men to replicate these results. Because age is the most important risk factor for AF, determining the relation of sex hormones in older men has important clinical utility. Second, our analysis cannot exclude residual confounding; in our discussion, we emphasized multiple pathways relating endogenous sex hormones and AF that were neither measured nor characterized by our analysis. Third, Framingham Heart Study participants may have unrecog-

association. Additional efforts are essential to replicate our analysis in larger cohorts. If validated, it will be essential to explore the electrophysiological and clinical mechanisms underlying the association between low testosterone and AF.

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Disclosures

None.

References

suggest the addition of AF as an end point in clinical trials evaluating the effects of testosterone repletion in older men. Extensively related to adverse outcomes, including heart failure and mortality, we appreciate establishing its relation to AF as clinical relevance because hypogonadism and AF are common in older men. Although hypogonadism in older men has been evaluated these associations. Finally, dehydroepiandrosterone levels were not associated with AF. We conclude that decreased levels of testosterone and AF, such that middle-aged men (55–69 years) had a 1.3-fold and older men (80 years) a 3.5-fold increased risk of AF for every 1 SD decrease in testosterone. A 1 SD decrease in baseline estradiol was associated with a hazard ratio of 1.12 (95% confidence interval, 1.01–1.26) for incident AF. Adjusting for interim heart failure during follow-up did not attenuate these associations. Finally, dehydroepiandrosterone levels were not associated with AF. We conclude that decreased levels of testosterone and estradiol are associated with increased risk of developing AF in middle-aged to older men. The finding has clinical relevance because hypogonadism and AF are common in older men. Although hypogonadism in older men has been extensively related to adverse outcomes, including heart failure and mortality, we appreciate establishing its relation to AF as novel. We consider identifying modifiable risk factors for AF as critical for prevention. Additional work is essential to replicate our results in larger cohorts and to examine the electrophysiological effects of hypogonadism that promote AF. Finally, we suggest the addition of AF as an end point in clinical trials evaluating the effects of testosterone repletion in older men.

CLINICAL PERSPECTIVE

We examined the association of endogenous sex hormones with risk of atrial fibrillation (AF) in the Framingham Heart Study. In 1251 men, we determined the relations of baseline levels of sex hormones testosterone, estradiol, and dehydroepiandrosterone with 10-year risk of AF in models adjusted for common AF risk factors. We identified an age interaction in the relation of testosterone and AF, such that middle-aged men (55–69 years) had a 1.3-fold and older men (80 years) a 3.5-fold increased risk of AF for every 1 SD decrease in testosterone. A 1 SD decrease in baseline estradiol was associated with a hazard ratio of 1.12 (95% confidence interval, 1.01–1.26) for incident AF. Adjusting for interim heart failure during follow-up did not attenuate these associations. Finally, dehydroepiandrosterone levels were not associated with AF. We conclude that decreased levels of testosterone and estradiol are associated with increased risk of developing AF in middle-aged to older men. The finding has clinical relevance because hypogonadism and AF are common in older men. Although hypogonadism in older men has been extensively related to adverse outcomes, including heart failure and mortality, we appreciate establishing its relation to AF as novel. We consider identifying modifiable risk factors for AF as critical for prevention. Additional work is essential to replicate our results in larger cohorts and to examine the electrophysiological effects of hypogonadism that promote AF. Finally, we suggest the addition of AF as an end point in clinical trials evaluating the effects of testosterone repletion in older men.
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**Supplementary Table 1.** Baseline participant characteristics and measures of endogenous sex hormones, total and unadjusted comparisons by the outcome of incident atrial fibrillation during 10-year follow-up.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>All Participants (n=1251)</th>
<th>Incident AF (n=275)</th>
<th>Without incident AF (n=976)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>68.0 ± 8.2</td>
<td>70.5 ± 7.0</td>
<td>67.3 ± 8.3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Smoking, present or prior</td>
<td>263 (21%)</td>
<td>55 (20%)</td>
<td>208 (21%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7 ± 3.8</td>
<td>26.8 ± 3.8</td>
<td>26.7 ± 3.7</td>
<td>0.73</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>138 ± 19</td>
<td>141 ± 20</td>
<td>138 ± 18</td>
<td>0.010</td>
</tr>
<tr>
<td>Hypertension treatment</td>
<td>436 (35%)</td>
<td>121 (44%)</td>
<td>315 (32%)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Diabetes</td>
<td>137 (11%)</td>
<td>36 (13%)</td>
<td>101 (10%)</td>
<td>0.20</td>
</tr>
<tr>
<td>PR interval duration, msec</td>
<td>174 ± 28</td>
<td>181 ± 36</td>
<td>172 ± 25</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Significant murmur*</td>
<td>61 (5%)</td>
<td>23 (8%)</td>
<td>38 (4%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Heart failure</td>
<td>23 (2%)</td>
<td>7 (3%)</td>
<td>16 (2%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Hormone levels†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone, ng/dl</td>
<td>450 (360, 560)</td>
<td>420 (330, 500)</td>
<td>470 (370, 570)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td>23.5 (15.2, 32.9)</td>
<td>21.9 (13.4, 30.6)</td>
<td>24.0 (15.6, 33.9)</td>
<td>0.005</td>
</tr>
<tr>
<td>DHEA-S, mcg/dl</td>
<td>112.0 (67.9, 173.0)</td>
<td>98.2 (59.6, 150.0)</td>
<td>115.0 (71.4, 181.0)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

DHEA-S, dehydroepiandrosterone sulfate. Continuous variables presented as mean ± standard deviation and categorical data as frequency (%). Estradiol and DHEA-S are depicted as median 25th%ile and 75th%ile due to their non-normal distribution.

* T-test for continuous variables and Chi-squared test for categorical variables.
† Wilcoxon rank sum test used.
Supplementary table 2. Association of 1-SD Endogenous Sex Hormone Decrease with 10-Year Risk of Incident Atrial Fibrillation, adjusting for interim heart failure.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Risk Estimate (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 55-69 years</td>
<td>1.30 (95% CI, 1.07 to 1.59)</td>
<td>0.009</td>
</tr>
<tr>
<td>Age 70-79 years</td>
<td>1.15 (95% CI, 0.91 to 1.45)</td>
<td>0.24</td>
</tr>
<tr>
<td>Age ≥80 years</td>
<td>3.85 (95% CI, 2.11 to 7.04)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estradiol</td>
<td>1.13 (95%CI, 1.01 to 1.26)</td>
<td>0.04</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>1.12 (95%CI, 0.98 to 1.27)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

All models were adjusted for smoking, body mass index, systolic blood pressure, hypertension treatment, diabetes, PR interval, significant murmur and prevalent heart failure, and interim heart failure during follow-up. SD, indicates standard deviation; CI, confidence interval; DHEA-S, dehydroepiandrosterone sulfate.