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Dehydroepiandrosterone (DHEA) and Its Sulfate (DHEA-S) Predict the Five-Year Risk of Coronary Heart Disease Events in Elderly Men

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Abstract

Objectives - We tested the hypothesis that serum DHEA and DHEA-S are predictors of major coronary heart disease (CHD) and/or cerebrovascular disease (CBD) events in a large cohort of elderly men.

Background - The adrenal sex hormone dehydroepiandrosterone (DHEA), present in serum mainly as the sulfate DHEA-S, is the most abundant steroid hormone in human blood and its levels decline dramatically with age. Despite a relatively large literature on vascular and metabolic actions of DHEA/-S, evidence for an association between DHEA/-S levels and cardiovascular events is contradictory.

Methods - We used gas/liquid chromatography-mass spectrometry to analyze baseline levels of DHEA and DHEA-S in the prospective population-based MrOS Sweden study (2416 men, 69-81 years). Complete cardiovascular clinical outcomes were available from national Swedish registers.

Results - During the 5-year follow-up, 302 participants experienced a CHD event and 225 a CBD event. Both DHEA and DHEA-S levels were inversely associated with the age-adjusted risk of a CHD event; hazard ratio and 95% confidence interval per SD increase was 0.82 (0.73-0.93) and 0.86 (0.77-0.97), respectively. By contrast, DHEA/-S showed no statistically significant association with the risk of CBD events. The association between DHEA and CHD risk remained significant after adjustment for traditional cardiovascular risk factors, serum total testosterone, estradiol, C-reactive protein and renal function and was unchanged after exclusion of the first 2.6 years of follow-up to reduce reverse causality.

Conclusions - Low serum levels of DHEA and its sulfate predict an increased risk of CHD, but not CBD, events in elderly men.

Abbreviations

ApoA1 = apolipoprotein A1

ApoB = apolipoprotein B

BMI = body mass index

CBD = cerebrovascular disease

CHD = coronary heart disease

CVD = cardiovascular disease

DHEA = dehydroepiandrosterone

DHEA-S = dehydroepiandrosterone sulfate

eGFR = estimated glomerular filtration rate

hsCRP = high sensitivity C-reactive protein

Introduction

The adrenal sex hormone dehydroepiandrosterone (DHEA) is the most abundant steroid hormone in human blood and is present in serum mainly as a sulfate ester (DHEA-S)(1, 2). DHEA may exert biological effects via peripheral conversion into sex steroids such as testosterone and estradiol, but other DHEA metabolites may also be biologically active and direct effects of DHEA have been proposed (1-3).

DHEA/-S levels decline dramatically with age (4); the mechanism behind this decline and its consequences for health are unclear (5). DHEA has been hypothesized to be of importance for body composition, insulin sensitivity and endothelial function (1, 4, 6), and DHEA may reduce vascular inflammation and remodeling, platelet aggregation, oxidative stress and atherosclerosis, suggesting that DHEA may confer vascular protection (1-3, 6-9). Consequently, it has been speculated that relative DHEA/-S deficiency with increasing age may contribute to age-related cardiometabolic disease (4). However, although there is a relatively large literature on the vascular and metabolic actions of DHEA in experimental models, the relevance of these results for human physiology and pathophysiology is uncertain (4).

Several studies that addressed the association between DHEA/-S levels and cardiovascular disease (CVD) outcomes showed inconsistent findings (6, 10-15). In prospective studies, we and others previously reported increased risk of both all-cause and CVD mortality among elderly men with the lowest DHEA/-S levels (16-18). However, the data regarding DHEA/-S and CVD mortality in men are also conflicting (19, 20) and may be confounded by the fact that DHEA/-S levels are suppressed in severe illness (21, 22). One prospective study reported

an association between low DHEA/-S and combined fatal and nonfatal coronary heart disease (CHD) events in men only when self-report of treated CHD and CHD medication were included as events in the analysis (23). Thus, there is a need for large population-based studies addressing the potential association between DHEA/-S and CVD outcomes.

The aim of the present study was to test the hypothesis that serum DHEA and/or DHEA-S levels are predictors of major CHD and/or cerebrovascular disease (CBD) events in a large population-based cohort of elderly men. We used state-of-the-art methodology to assay baseline DHEA and DHEA-S serum levels, and had a complete 5-year follow up for CHD (including hospitalization for acute myocardial infarction, unstable angina or revascularization, or death from CHD) and CBD (including hospitalization for stroke or transient ischaemic attack, or death from stroke) endpoints.

Methods

Study Population

The multicenter Osteoporotic Fractures in Men (MrOS) Study includes older men in Sweden, Hong Kong, and the United States. Swedish study participants (69–81 years) were randomly selected from national population registers (24). Eligibility required the ability to walk without walking aids, ability to provide self-reported data, and to understand and sign an informed consent; 45% of those contacted participated in The MrOS Study in Sweden (n=3014), which includes cohorts in three cities: Malmö (n=1005), Göteborg (n=1010), and Uppsala (n=999). The study was approved by the ethics committees at Göteborg, Lund, and Uppsala Universities.

We investigated the associations between serum DHEA and DHEA-S with CHD/CBD events in the Swedish MrOS cohort. Serum samples were drawn in the morning (before 10 a.m.; 69% of the cohort) or around noon (between 10 a.m. and 3 p.m., average 1 p.m.; 31%). A sufficient amount of serum for assessment of DHEA by gas chromatography-mass spectroscopy was available for 2639 men (99% of the participants in the Göteborg cohort, 96% in the Malmö cohort, and 68% in the Uppsala cohort). Of these, 223 participants were excluded for the following reasons; treatment with testosterone, 5alpha-reductase inhibitors, gonadotrophin releasing hormone agonists or antiandrogens or a history of surgical castration. This left 2416 men for the present analyses.

Assessment of Covariates

We used a standardized questionnaire (25) to gather information about smoking habits and self-reported medical diagnoses (including hypertension, diabetes, and myocardial infarction). Diabetes was defined as a self-reported medical diagnosis of diabetes. Hypertension was

defined as a self-reported medical diagnosis with either self-reported anti-hypertensive treatment or a supine systolic blood pressure of ≥140 mmHg (measured after 10 min rest). Standard equipment was used to measure height and weight; body mass index (BMI) was calculated as (kg/m^2) =weight (kg)/height $(m)^2$.

Apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1) were determined by immunoprecipitation enhanced by polyethylene glycol at 340 nm (Thermo Fisher Scientific, Vantaa, Finland). High sensitivity C-reactive protein (hsCRP) was measured by an ultrasensitive method (Orion Diagnostica, Espoo, Finland). Both analyses were performed on a Konelab 20 autoanalyzer (Thermo Fisher Scientific). Inter-assay coefficient of variation (CV) for the Konelab analyses were below 5%.

For assessment of estimated glomerular filtration rate (eGFR), Cystatin C was analyzed with polyclonal antibodies against human cystatin C and measured by immunoturbidimetry (Cystatin C Immunoparticles; Dako Denmark A/S, Glostrup, Denmark). The eGFR was calculated using the following formula: GFR = 79.901 * (cystatin C)−1.4389. This proxy for GFR has good precision, good linearity, and strong correlation with iohexol clearance (R^2 = 0.956)(26).

Serum Sex Steroids

All samples were frozen at -80^{o}C and shipped on dry ice to one laboratory (Laboratory of Molecular Endocrinology, Laval University Hospital Research Center, Québec City, Québec, Canada). A validated gas chromatography-mass spectroscopy system (27-29) was used to analyze DHEA (limit of detection, 0.20 ng/ml; intra-assay CV, 2.0%; inter-assay CV, 1.9%), testosterone (limit of detection, 0.05 ng/ml; intra-assay CV, 2.9%; inter-assay CV, 3.4%) and

estradiol (limit of detection, 2.00 pg/ml; intra-assay CV, 1.5%; inter-assay CV, 2.7%), using a 50% phenyl-methyl polysiloxane (DB-17HT) capillary column (30 m × 0.25 mm internal diameter; 0.15-μm film thickness) with helium as carrier gas. The analytes and the internal standard were detected using a HP5973 quadrupole mass spectrometer equipped with a chemical ionization source. DHEA-S (limit of detection, 0.075 μg/ml; intra-assay CV, 5.2%; interassay CV, 6.3%) was analyzed by a validated liquid chromatography-tandem mass spectroscopy method using TurboIonSpray^{TM}, as previously described (27). To measure serum sex hormone-binding globulin, we used an immunoradiometric assay (Orion Diagnostics, Espoo, Finland; limit of detection, 1.3 nmol/l; intra-assay CV, 3%; interassay CV, 7%).

Study Outcomes

Follow-up time was the period between baseline visit (in 2001-2004) and date of death, first CHD/CBD event or last data collection (December 31, 2008). Cause of death data were collected from the Swedish Causes of Death Register, held by the National Board of Health and Welfare in Sweden, in which all deaths in Sweden are registered with International Classification of Diseases (ICD) codes based on information from death certificates. Data were collected from this register from study start until December 31, 2005 and from evaluation of copies of death certificates for deaths occurring after this date up to 2008. Based on the information from the register/death certificate, the underlying cause of death was determined and classified for each participant; CHD death was defined by ICD-10 codes I20 to I25 and stroke death by I60-I64. Data on hospitalization for first acute myocardial infarction (ICD-10 codes I21-I23), unstable angina (ICD-10 codes I20.0 and I24), revascularization procedure (surgery code FN), stroke (ICD-10 codes I60-I64) or transient ischaemic attack (ICD-10 code G45) were collected from the Swedish Hospital Discharge

Register between the baseline date and December 31, 2008. The combination of data from the Swedish Causes of Death Registry and the Swedish Hospital Discharge Register has been shown to be an efficient, validated alternative to hospital discharge notes and death certificates for both CHD and stroke (30). CHD events were defined as a composite endpoint of hospitalization for acute myocardial infarction, unstable angina or revascularization, or death from CHD. CBD events included hospitalization for stroke or transient ischaemic attack, or death from stroke. Death due to other causes than CHD (for the analyses of CHD events) or stroke (for the analyses of CBD events) resulted in censoring in the analyses.

Statistical Analysis

The associations between serum log transformed DHEA and DHEA-S, as well as serum DHEA/-S and total testosterone, estradiol, and sex hormone-binding globulin (log transformed) was tested by Pearson correlation. Covariates of serum DHEA/-S were studied in a multiple regression model with log-transformed DHEA/-S as the dependent variable and age, morning sample (yes/no), MrOS site (dummy-coded), BMI (log transformed), ApoB/A1 ratio, serum hsCRP (log transformed), eGFR, current smoking, diabetes and hypertension as independent variables.

We used Cox proportional hazards regression to analyze the associations between serum DHEA and DHEA-S and CVD outcomes, i.e. first CHD or CBD event, respectively. The proportional hazard function was tested (by method developed by Therneau and Grambsch) and graphically assessed based on Schoenfeld residuals for both CHD and CBD models. No systematic deviations from the proportionality was detected. We show the effect estimate for a 1 SD increase (z score) of log transformed DHEA/-S levels. All estimates were adjusted for age and morning sample (yes/no). Further, all Cox analyses were adjusted for MrOS site

(dummy-coded) and there were similar associations between DHEA (per SD) and CHD events across the three MrOS sites (data not shown). Further adjustments were made for BMI (log transformed), ApoB/A1 ratio, serum hsCRP (log transformed), serum total estradiol and testosterone, serum sex hormone-binding globulin (log transformed) and eGFR as continuous variables and three dichotomous variables: current smoking, diabetes and hypertension. We also examined the associations between DHEA/-S and outcomes across the components of the CHD composite endpoint.

Unadjusted Kaplan-Meier survival curves illustrated the association between tertiles of DHEA and CHD as well as CBD events and the log-rank test assessed statistical significance. Possible non-linearity in the association between DHEA and CHD outcomes was tested by entering DHEA/-S as quadratic terms in the Cox regression analyses. Further, in the Cox regression analysis we used a restricted cubic spline-approach for a flexible non-linear assessment of the hazard ratio (HR) in relation to DHEA (31). The positions and the number of knots were selected using the Akaike Information Criterion (32). Five knots positioned at the percentiles 10, 25, 50, 75 and 90 of log-transformed serum DHEA concentration was found to give a small AIC and to capture the average curve shape over a systematical assessment of different alternatives. In the analysis using a spline approach, age and MrOS site were entered as covariates.

We performed the restricted cubic spline analysis using SAS version 9.2 (SAS Institute, Cary, NC, USA) and other statistical analyses using SPSS for Windows (version 19.0; SPSS, Chicago, IL).

Results

At baseline, the mean age of the cohort (n=2614) was 75.4 years (Table 1). Serum levels of DHEA and DHEA-S in the cohort were highly collinear (r=0.73, P<0.001), but their covariates differed slightly (Table 2A-B). The covariates of DHEA and DHEA-S levels in a multiple regression model (Table 2B) were age, BMI, hsCRP and diabetes. Besides these factors, DHEA, but not DHEA-S, levels were also highly influenced by time of blood sampling and renal function.

During follow-up, a total of 302 participants experienced a CHD event (rate of 2.5 per 100 person-years at risk) and 225 participants had a CBD event (1.9 per 100 person-years at risk). The median follow-up time (to death, first event or last data collection) was 5.2 yrs (12.070 person-years) for CHD events and 5.2 years (12.137 person-years) for CBD events. Except for 3 participants who moved abroad, there was no loss of follow-up.

In prospective analyses, DHEA and DHEA-S levels were both inversely associated with the age-adjusted risk of CHD events when analyzed as continous variables (Table 3; Model 1). By contrast, there was no statistically significant association between DHEA or DHEA-S and CBD events in corresponding analyses (Table 3). After adjustment for traditional cardiovascular risk factors (age, BMI, smoking, diabetes, hypertension and ApoB/A1ratio; Model 2), the associations between DHEA/-S and CHD risk remained significant (Table 3). Spline and quadratic models did not support a non-linear association between DHEA level and CHD risk (Figure 1 and data not shown).

Given that DHEA/-S may modulate immune responses (7), we addressed inflammation as a potential mediator between lower DHEA/-S and increased CHD risk. Adjustment of the

association for hsCRP level did not materially change the association between DHEA/-S and CHD risk (Table 3).

Both DHEA and DHEA-S levels were directly associated with renal function assessed by eGFR (Table 2A). Therefore, we adjusted the association between DHEA/-S and CHD risk for eGFR and found the point estimates for CHD risk not materially changed (Table 3).

DHEA/-S is a precursor hormone for testosterone and estradiol (4). DHEA showed an association with serum testosterone ($r=0.21$, $P<0.001$) and estradiol ($r=0.14$, $P<0.001$) that was slightly stronger than the association between DHEA-S and testosterone ($r=0.05$, $P=0.010$) and estradiol ($r=0.06$, $P=0.005$), respectively. Following adjustment of the association between low DHEA/-S and CHD risk for serum total testosterone and estradiol, the associations remained unchanged (Table 3).

We also examined the association between DHEA/-S and sex hormone-binding globulin, which has an important role in sex hormone biology (33). Sex hormone-binding globulin levels were significantly associated with DHEA-S ($r=-0.09$, $p<0.001$) but not with DHEA ($r=0.03$, $p=0.095$). Adjustment for sex hormone-binding globulin level did not change the association between DHEA/-S and CHD events (Table 3).

In a subanalysis, we examined the associations between DHEA/-S and outcomes across the components of the CHD composite endpoint (Table S1). We found that the point estimates for the DHEA/-S association with CHD death, hospitalization for MI, hospitalization for unstable angina, and hospitalization for revascularization all are in the same direction, with the

strongest point estimates for CHD death and hospitalization for unstable angina and the weakest point estimates for revascularization procedures.

To illustrate the association between DHEA status and CVD risk, we plotted unadjusted Kaplan-Meier curves of CHD and CBD event-free survival stratified by DHEA tertiles. These plots illustrate that men with relatively lower DHEA had statistically significantly increased risk of a CHD event (Figure 2A), but not a CBD event (Figure 2B).

DHEA levels are suppressed in severe illness (21, 22), and general health status is therefore a potential confounder of observed associations. To study the role of subclinical diseases, we performed an analysis excluding men with a short follow-up time indicative of poor health at the baseline examination. After exclusion of men with a follow-up of 2.6 years or less (half of the median follow-up time), the age-adjusted association between low DHEA levels and CHD risk was not attenuated (Table 4). Similarly, the association between DHEA and CHD risk remained following exclusion of men with reduced renal function (eGFR $\leq$45 mL/min/1.73 m^2) as well as men with non-morning samples. Further, the association between DHEA and CHD risk remained after exclusion of men with a baseline history of myocardial infarction.

Discussion

In the present large, population-based cohort study of elderly men followed for 5 years, we found that baseline serum levels of DHEA and DHEA-S predicted future CHD events. By contrast, DHEA/-S showed no statistically significant association with the risk of CBD events. The association between DHEA and CHD risk remained significant after adjustment for traditional cardiovascular risk factors, serum testosterone and estradiol levels, hsCRP and renal function. Further, the association between DHEA and CHD risk was not materially changed in analyses excluding the first 2.6 years of follow-up.

Previous epidemiological evidence of an association between DHEA/-S levels and CVD outcomes in men are contradictory (10-20, 23, 34). A prospective nested case-control study reported lower DHEA-S among fatal CHD cases (10) but several smaller prospective case-control studies of fatal/non-fatal CHD events (13-15) found no association with DHEA-S level. In larger prospective cohort studies, we and others previously found increased risk of both all-cause and CVD mortality among elderly men with the lowest DHEA/-S levels (16-18), but other large studies found no association between DHEA-S and CVD mortality in men (19, 20). Further, a population-based study reported no association between DHEA-S levels and incident CVD in 2084 middle-aged (mean age 55 years) men (34). The Massachusetts Male Aging Study found no significant association between low DHEA/-S and the 9-year CHD mortality among 1,167 men aged 40-70 years, but did find an association between low DHEA/-S and combined fatal and non-fatal CHD events (151 events), however only when self-report of treated CHD and CHD medication were included as events in the analysis (23). Taken together, previous data are conflicting, and there is a paucity of large prospective analyses on this topic. Our study represents the largest study so far (302 men with CHD

events and 225 with CBD events in 2,416 men at risk), and strongly supports an association between DHEA/-S levels and CHD risk in (elderly) men.

Experimental *in vitro* and rodent studies suggest that DHEA/-S may modulate lipid/glucose metabolism, systemic inflammation and vascular endothelial function and vascular remodeling via different mechanisms, such as PPARα activation, activation of a G protein-coupled receptor or conversion to downstream DHEA metabolites (1-3, 9). However, because adult rodents do not produce DHEA/-S in measurable amounts due to lack of expression of the enzyme CYP17 in the adrenals (35), results from rodent studies must be interpreted with caution. Short-term trials on the effect of DHEA therapy on vascular endothelial function in humans have reported both improvement (36, 37) and no effect (38, 39). Similarly, four longer (6 months-2 years) trials of DHEA therapy in elderly persons show conflicting results on the effects on body composition and insulin action (40-44). In the present study, adjustment for traditional vascular risk factors did not affect the association between DHEA/-S and CHD risk, arguing against a mechanism involving these. Moreover, our results do not support systemic inflammation as a mediator as adjustment for hsCRP level had no impact. Further, we found that the association between DHEA/-S and CHD risk was not materially changed following adjustment for low serum testosterone and estradiol levels. Importantly, this does not exclude a pivotal role for androgens or estrogens that are produced locally from the metabolism of DHEA (4). The findings of the present study should encourage further mechanistic studies, preferrably performed in humans or other primates. These studies should particularly address possible direct actions of DHEA on the vascular wall, *e.g.* on endothelial function/regeneration (2, 37, 45, 46) and proliferation of vascular smooth muscle cells (3, 47, 48).

Although collinear, the covariates of DHEA and DHEA-S differed slightly. In line with previous findings that DHEA secretion follows a diurnal rhythm similar to that of cortisol (2, 16), DHEA associated with time of blood sampling, while DHEA-S did not, as expected from the significantly longer half-life of DHEA-S (2). Importantly, DHEA, but not DHEA-S, was associated with renal function in the multivariable models. It is conceivable that a "low DHEA status" is not properly reflected by DHEA-S levels, especially when renal function is reduced, because of reduced renal clearance of the conjugated (sulfated), and thereby water soluble, form of the hormone. Thus, the impact of any systemic disease on DHEA-S levels may be counteracted by concomitant renal dysfunction (49). Nevertheless, adjustment for renal function had no major impact on the association between DHEA or DHEA-S and CHD risk in our study.

In the present study, both DHEA and DHEA-S blood levels predicted CHD events. The relative biological importance and pathways of DHEA and DHEA-S are unclear, and the collinearity between DHEA and DHEA-S precludes firm conclusions from statistical analyses. The concentration of circulating DHEA is about 350 times lower than that of DHEA-S, but DHEA may be equally important, because it is more readily converted to downstream tissue metabolites (1, 2). Other data suggest that DHEA-S is back-converted to DHEA only to a minor degree (2, 50). The sulfation and desulfation of DHEA are actively catalyzed by sulfotransferase and sulfatase, respectively, which may be regulated by different intra-individual and/or environmental factors and disease states (51, 52). Gaining further insight into the biological role of the conjugated versus non-conjugated form of DHEA and how DHEA sulfation is regulated are important tasks for future studies.

As any systemic disease and general poor health may lower DHEA/-S (22), comorbidity might explain associations between DHEA/-S and CHD events. Those who have poor general health (of any cause) at baseline are more likely to die and/or experience a CHD or non-CHD event soon after baseline. Therefore, our finding that the association between DHEA and CHD risk was unchanged following exclusion of the first 2.6 years of follow-up argues against comorbidity confounding the observed association.

The results of several small DHEA supplementation studies are inconclusive (1). Although available data does not support DHEA supplementation to elderly people, there is a widespread, non-supervised use of DHEA as a dietary supplement. Our results highlight the need for further larger trials of the cardiometabolic consequences of DHEA therapy in elderly subjects and/or subjects with low DHEA/-S levels.

If DHEA/-S is cardioprotective, drugs that target the synthesis of DHEA/-S may adversely affect CHD risk. Of note, abiraterone acetate, which inhibits the enzyme CYP17 and thereby the biosynthesis of both DHEA and testosterone, is a new therapy for advanced prostate cancer. Abiraterone acetate was recently shown to increase short-term overall survival in patients with metastatic prostate cancer (53), but concerns about potential cardiovascular side effects may be raised due to its pronounced DHEA-lowering effect.

Our study has limitations; the results are based on single measurements of DHEA and DHEA-S and may underestimate true associations. Given the diurnal variation in serum DHEA levels (16), the use of some non-morning samples may contribute to increased variability, but the hour of day was adjusted for in the analyses and deleting the participants without AM samples did not materially change the results. Another limitation is that baseline covariates were partly

self-reported, which is a potential source of residual confounding. Older adults are also being treated with more CVD medications some of which alter cardiovascular risk and/or DHEA/-S levels. The study also has notable strengths, including the mass spectrometry DHEA/-S methodology, a large well-characterized sample, complete follow-up, fatal and nonfatal outcomes, and the documented accuracy of classification in nationwide Swedish registers (30).

In conclusion, low serum levels of DHEA and its sulfate predict the risk of CHD, but not CBD, events in elderly men.

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Figure legends

Figure 1. Smoothed plots of log-transformed HR for CHD events according to serum DHEA concentration. DHEA levels were log_{10}-transformed; corresponding serum levels are shown in italics. HRs (red solid line) and 95% CIs (blue dotted lines) were estimated by restricted cubic spline Cox regression analysis, using the median serum DHEA concentration (1.5 ng/mL) as the reference value. Five knots were positioned at the 10th, 25th, 50th, 75th, and 90th percentiles of serum logDHEA concentration. The horizontal dashed line corresponds to the reference (1.5 ng/mL) HR of 1.0. The model is adjusted for age and MrOS site. P(effect)=0.001; P(nonlinearity)=0.1.

Figure 2. Unadjusted Kaplan-Meier curves of event-free survival by serum DHEA tertile for CHD and CBD events. A, total number of participants with CHD events=302; B, total number of participants with CBD events=225. Blue is low (tertile 1), green is intermediate (tertile 2), and yellow is high (tertile 3) serum DHEA concentration. P-value for trend over tertiles assessed by log-rank test was P=0.015 for CHD and P=0.059 for CBD events, respectively. CHD indicates coronary heart disease; CBD, cerebrovascular disease.

Tables

Table 1. Characteristics of the Study Participants

Variable	
No.	2416
Age (years)	75.4 ± 3.2
BMI (kg/m^2)*	26.1 ± 1.1
ApoB/A1	0.72 ± 0.20
hsCRP (mg/L)*	2.63 ± 2.36
Current smoking (%)	8.4
Diabetes (%)	9.4
Hypertension (%)	34.3
eGFR (mL/min/1.73 m^2)	71.9 ± 20.5
DHEA (ng/mL)	1.79 ± 1.20
DHEA-S (µg/mL)	0.71 ± 0.46
Testosterone (ng/mL)	4.53 ± 1.73
Estradiol (pg/mL)	21.1 ± 7.5
SHBG (nmol/L)	44 ± 22

BMI indicates body mass index; ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; hsCRP, high sensitivity C-reactive protein; DHEA , dehydroepiandrosterone; DHEA-S, DHEA sulfate; eGFR, estimated glomerular filtration rate; SHBG, sex hormone-binding globulin.

Diabetes was defined as a self-reported medical diagnosis of diabetes. Hypertension was defined as a self-reported medical diagnosis with either self-reported anti-hypertensive treatment or a supine systolic blood pressure of ≥140 mmHg.

Values are given as mean±SD unless otherwise indicated.

* Geometric mean±SD

Table 2. Covariates of DHEA and DHEA-S Levels

A. Univariate Correlations

Variable	DHEA*		DHEA-S*	
	r	*P*	*r*	*P*
Morning sample†	0.24	<0.001	-0.01	0.51
Age	-0.22	<0.001	-0.19	<0.001
BMI*	-0.10	<0.001	-0.05	0.011
ApoB/A1	-0.04	0.047	-0.02	0.25
hsCRP*	-0.11	<0.001	-0.07	<0.001
Current smoking†	0.04	0.026	0.05	0.008
Diabetes†	-0.06	0.004	-0.07	<0.001
Hypertension†	-0.05	0.011	-0.03	0.10
eGFR	0.15	<0.001	0.09	<0.001

B. Multivariate Correlations

Variable	DHEA*		DHEA-S*	
	Standardized Beta Coefficient	*P*	*Standardized Beta Coefficient*	*P*
Morning sample†	0.22	<0.001	0.01	0.68
Age	-0.19	<0.001	-0.20	<0.001
BMI*	-0.09	<0.001	-0.05	0.013
ApoB/A1	0.04	0.072	-0.02	0.43
hsCRP*	-0.07	<0.001	-0.06	0.009
Current smoking†	0.04	0.046	0.04	0.064
Diabetes†	-0.05	0.010	-0.06	0.002
Hypertension†	-0.01	0.60	-0.00	0.85
eGFR	0.08	<0.001	0.02	0.31

BMI indicates body mass index; ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; hsCRP, high sensitivity C-reactive protein; DHEA , dehydroepiandrosterone; DHEA-S, DHEA sulfate; eGFR, estimated glomerular filtration rate.

*Log_{10}-transformed values of DHEA, DHEA-S, BMI and hsCRP were used.

†yes=1, no=0 for dichotomous variables

Table 3. Adjusted Hazard Ratios of DHEA and DHEA-S as Continuous Variables for CHD and CBD Events

	No. of events/No. of subjects	DHEA HR (95% CI)	DHEA-S HR (95% CI)
CHD Events			
Per SD increase; Model 1	302/2416	0.82 (0.73-0.93) P=0.001	0.86 (0.77-0.97) P=0.011
Per SD increase; Model 2	290/2341	0.83 (0.73-0.94) P=0.002	0.87 (0.78-0.98) P=0.022
Per SD increase; Model 2 + hsCRP	290/2341	0.84 (0.74-0.95) P=0.005	0.88 (0.78-0.99) P=0.030
Per SD increase; Model 2 + eGFR	289/2324	0.85 (0.75-0.96) P=0.007	0.88 (0.78-0.99) P=0.031
Per SD increase; Model 2 + estradiol and testosterone	290/2341	0.83 (0.73-0.94) P=0.003	0.87 (0.78-0.98) P=0.020
Per SD increase; Model 2 + SHBG	290/2341	0.83 (0.74-0.94) P=0.003	0.87 (0.77-0.97) P=0.015
CBD Events			
Per SD increase;Model 1	225/2416	0.91 (0.79-1.04) P=0.17	0.90 (0.79-1.02) P=0.11
Per SD increase; Model 2	222/2341	0.90 (0.79-1.04) P=0.16	0.90 (0.79-1.02) P=0.10

Model 1: adjustment for age, MrOS site, morning sample.
Model 2: Model 1 + current smoking, BMI, diabetes, hypertension, ApoB/A1

CHD indicates coronary heart disease; CBD, cerebrovascular disease; BMI, body mass index; ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; hsCRP, high sensitivity C-reactive protein; DHEA , dehydroepiandrosterone; DHEA-S, DHEA sulfate; eGFR, estimated glomerular filtration rate; SHBG, sex hormone-binding globulin.

Log_{10}-transformed values of DHEA, DHEA-S, BMI and hsCRP were used.

The proportional hazards assumption was met for both CHD and CBD models.

Table 4. Hazard Ratios of DHEA for CHD Events: Exclusion of Subgroups

	No. of CHD events/ No. of subjects	DHEA HR (95% CI)
Per SD increase; Model 1 (From Table 3)	*302/2416*	*0.82 (0.73-0.93) P=0.001*
Per SD increase; Model 1 Exclusion of men with a follow-up time of 2.6 yrs* or less (n=215)	162/2201	0.80 (0.68-0.94) P=0.007
Per SD increase; Model 1 Exclusion of men with reduced renal function (eGFR≤45 mL/min/1.73 m^2; n=241)	260/2175	0.86 (0.75-0.97) P=0.019
Per SD increase; Model 1 Exclusion of men with non-morning samples (n=750)	208/1666	0.84 (0.73-0.96) P=0.014
Per SD increase; Model 1 Exclusion of men with a baseline history of myocardial infarction (n=344)	211/2072	0.85 (0.74-0.97) P=0.019

Model 1: adjustment for age, MrOS site, morning sample.

Log_{10}-transformed values of DHEA were used.

*Half of the median follow-up time

Fig 1

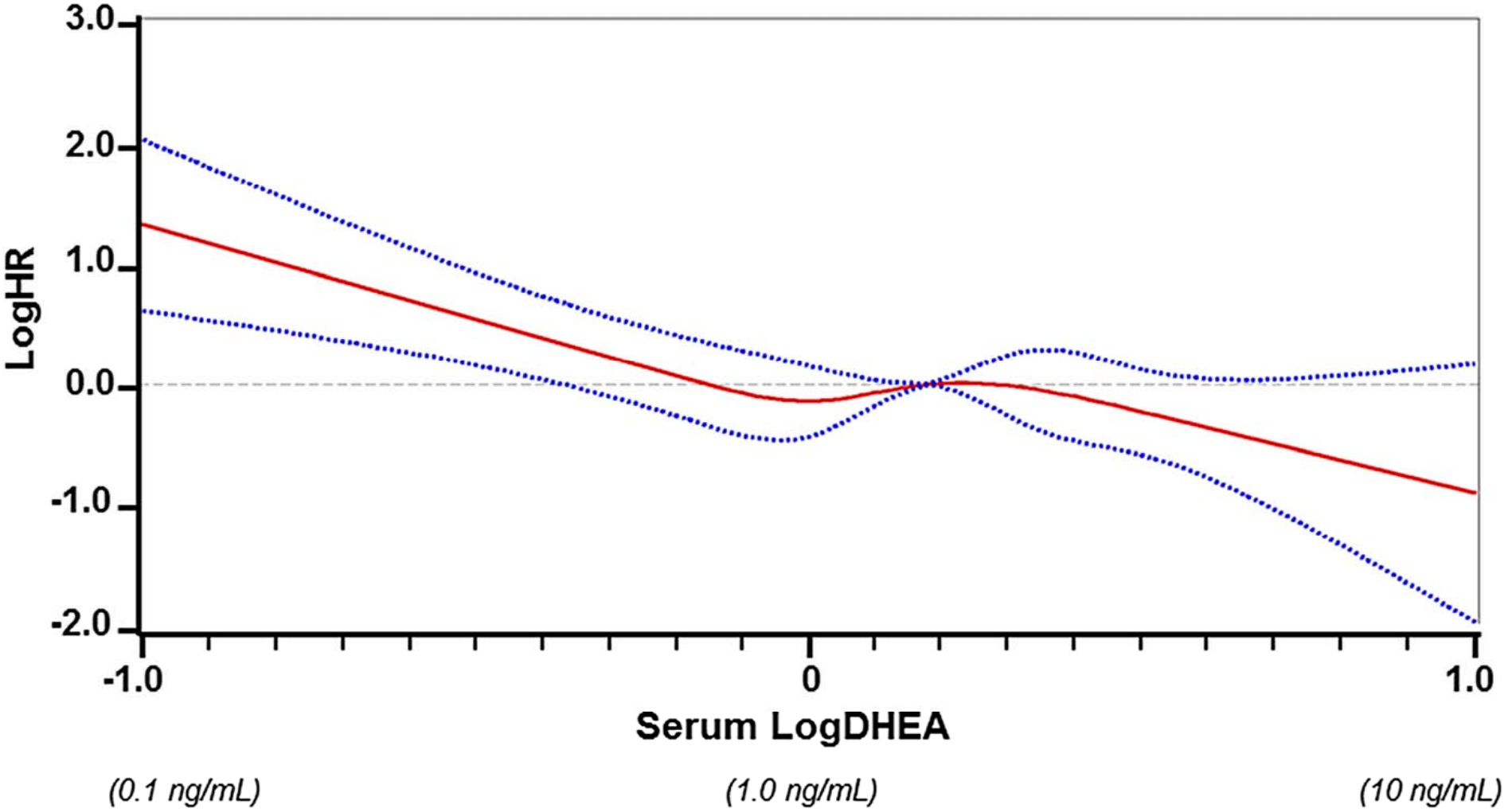
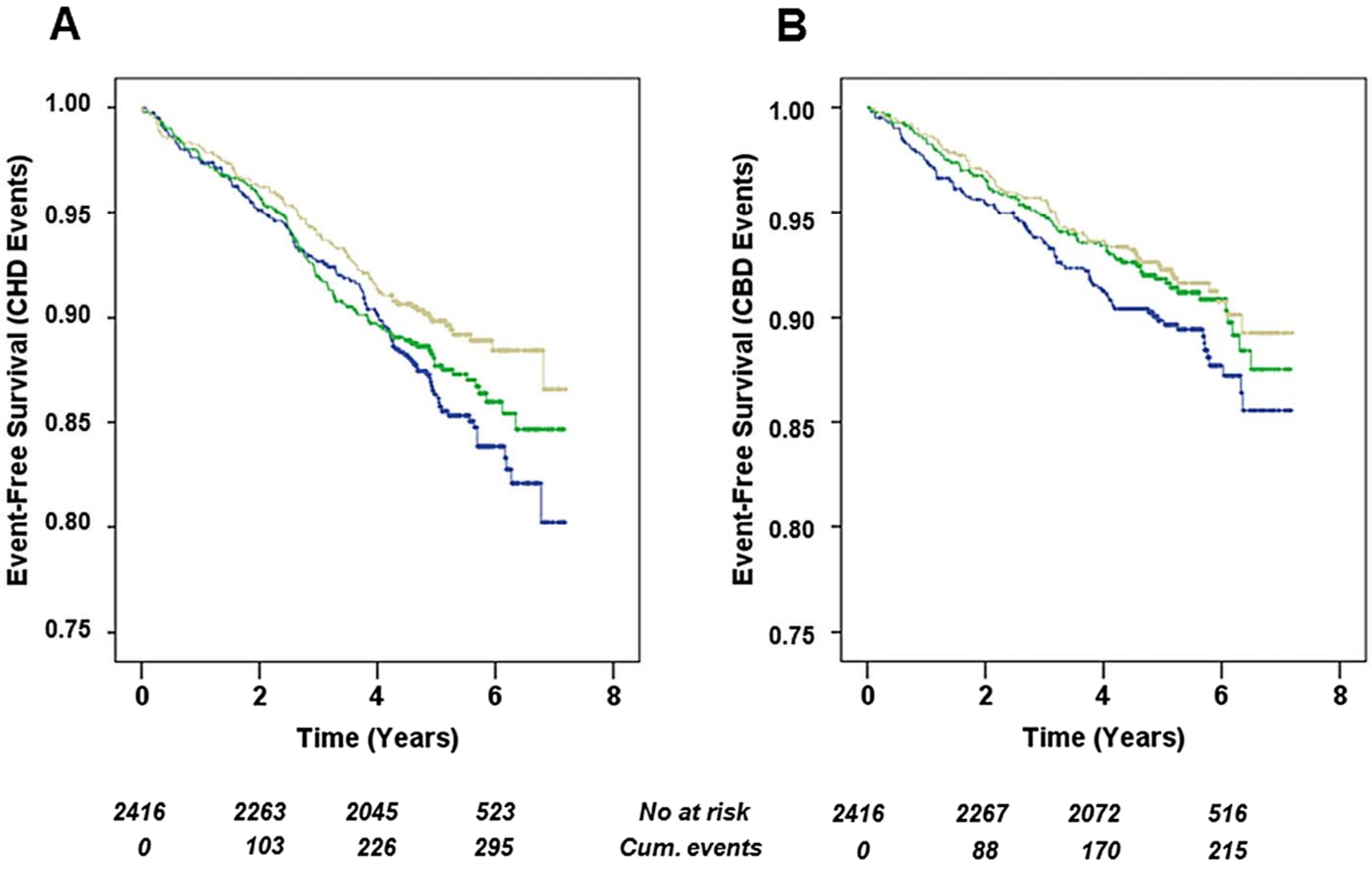

3.0
2.0
1.0
0.0
-1.0
-2.0
LogHR
-1.0
0
1.0
Serum LogDHEA
(0.1 ng/mL)
(1.0 ng/mL)
(10 ng/mL)

Fig 2


A
B
Event-Free Survival (CHD Events)
Event-Free Survival (CBD Events)
1.00
0.95
0.90
0.85
0.80
0.75
0
2
4
6
8
Time (Years)
No at risk
Cum. events
2416 2263 2045 523
0 103 226 295
2416 2267 2072 516
0 88 170 215

Supplemental Table

Table S1. Hazard Ratios of DHEA/-S for CHD Events: Associations Across the Components of the CHD Composite Endpoint

	No. of events/ No. of subjects	DHEA HR (95% CI)	DHEA-S HR (95% CI)
*All CHD events**	*302/2416*	*0.82 (0.73-0.93)*	*0.86 (0.77-0.97)*
Per SD increase; Model 1		*P=0.001*	*P=0.011*
(From Table 3)			
Death from CHD	88/2416	0.75 (0.61-0.92)	0.74 (0.61-0.90)
Per SD increase; Model 1		P=0.005	P=0.003
Hospitalization for myocardial infarction	164/2416	0.89 (0.76-1.04)	0.94 (0.80-1.10)
Per SD increase; Model 1		P=0.14	P=0.43
Hospitalization for unstable angina	66/2416	0.76 (0.60-0.97)	0.72 (0.58-0.90)
Per SD increase; Model 1		P=0.025	P=0.005
Hospitalization for revascularization	157/2416	0.93 (0.79-1.10)	0.95 (0.81-1.12)
Per SD increase; Model 1		P=0.38	P=0.53

DHEA indicates dehydroepiandrosterone; DHEA-S, DHEA sulfate; CHD, coronary heart disease
Model 1: adjustment for age, MrOS site, morning sample.
Log_{10}-transformed values of DHEA/-S were used.
*First CHD event during follow-up was analyzed