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Low Serum Levels of Dehydroepiandrosterone Sulfate Predict All-Cause and Cardiovascular Mortality in Elderly Swedish Men

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Context: The age-related decline in dehydroepiandrosterone (DHEA) levels is thought to be of importance for general and vascular aging. However, data on the association between DHEA and mortality are conflicting.

Objectives: We tested the hypothesis that low serum DHEA and DHEA sulfate (DHEA-S) levels predict all-cause and cardiovascular disease (CVD) death in elderly men.

Design, Setting, and Participants: We used gas/liquid chromatography-mass spectrometry to analyze baseline levels of DHEA and DHEA-S in the prospective population-based MrOS Sweden study (2644 men, aged 69–81 yr). Mortality data were obtained from central registers and analyzed using Cox proportional hazards regressions.

Main Outcome Measures: All-cause and CVD mortality by serum DHEA(-S) levels.

Results: During a mean 4.5-yr follow-up, 328 deaths occurred. Low levels of DHEA-S (quartile 1 vs. quartiles 2–4), predicted death from all causes [hazard ratio (HR) 1.54, 95% confidence interval (CI) 1.21–1.96; adjusted for traditional cardiovascular risk factors], from CVD ($n = 123$ deaths; HR 1.61, 95% CI 1.10–2.37) and ischemic heart disease ($n = 73$; HR 1.67, 95% CI 1.02–2.74) but not cancer. Analyses with DHEA gave similar results. The association between low DHEA-S and CVD death remained after adjustment for C-reactive protein and circulating estradiol and testosterone levels. When stratified by the median age of 75.4 yr, the mortality prediction by low DHEA-S was more pronounced among younger (age adjusted HR for CVD death 2.64, 95% CI 1.37–5.09) than older men (HR 1.30, 95% CI 0.83–2.04).

Conclusions: Low serum levels of DHEA(-S) predict death from all causes, CVD, and ischemic heart disease in older men. (*J Clin Endocrinol Metab* 95: 0000–0000, 2010)

Dehydroepiandrosterone (DHEA) is a sex steroid hormone from the adrenal cortex that circulates at high serum levels (1). The current concept is that DHEA exerts biological effects via conversion into active sex steroids (*e.g.* testosterone and estradiol) within peripheral target

tissues (1). Thus, the effects of DHEA are highly dependent on the local activity of DHEA-metabolizing enzymes, which are expressed and regulated in a cell type-specific fashion (1). DHEA may also exert direct effects, *e.g.* via activation of the peroxisome proliferator-activated re-

ceptor- α (2) and/or a yet-unidentified membrane receptor (2). DHEA has been hypothesized to be of importance, *e.g.* for bone physiology, body composition and insulin sensitivity (1, 3, 4), immune functions (5–7) and vascular physiology (2, 3).

DHEA is the principal form of the steroid hormone precursor, but it is mainly present in serum as a sulfate ester [DHEA sulfate (DHEA-S)], which can be desulfated to DHEA in a wide range of tissues (2). DHEA-S has a longer half-life in blood than DHEA, and, unlike DHEA, DHEA-S blood levels show little diurnal variation (2). DHEA-S, rather than DHEA, has been used in both clinical practice and most epidemiological studies. Few epidemiological studies have simultaneously assessed the predictive value of DHEA and DHEA-S for clinical outcomes.

DHEA and DHEA-S levels decline dramatically with age, such that levels in the oldest old are only 10–20% of the maximal levels observed between 20 and 30 yr of age (1). Given its multiple metabolic effects, this decline in DHEA levels has been thought to play a role in the aging process (1). The results of several small DHEA supplementation studies are inconclusive; nevertheless, there is a widespread, nonsupervised use of DHEA as a dietary supplement for elderly people (4).

Supporting the notion of a role in the ageing process, several cohort studies show an association between low DHEA-S and increased all-cause (8–12) and cardiovascular disease (CVD) (8, 9) mortality in men. This association is generally not found in women (3, 9). However, even among men, the results of studies of low DHEA-S and all-cause or CVD mortality are conflicting (13–17).

The aim of the present investigation was to study DHEA and DHEA-S as predictors of all-cause, CVD, and ischemic heart disease (IHD) death in a large, well-characterized, population-based cohort of older men. We used specific gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry, respectively, to assess baseline DHEA and DHEA-S levels in serum and had a 100% follow-up for vital status for 4.5 yr.

Subjects and Methods

Study population

The multicenter Osteoporotic Fractures in Men (MrOS) Study includes older men in Sweden, Hong Kong, and the United States. In Sweden, study participants (men aged 69–81 yr) were randomly selected from national population registers (18). Eligibility for study participation required the ability to walk unassisted by another person, provide self-reported data, and understand and sign an informed consent and not have bilateral hip prostheses; 45% of those contacted participated in the MrOS Study in Sweden ($n = 3014$), which includes three subcohorts 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 here the associations between serum DHEA, DHEA-S, and mortality in the Swedish MrOS cohort. Levels of DHEA were available for 2639 men and DHEA-S for 2644 men (99% of the participants in the Göteborg cohort, 96% in the Malmö cohort, and 68% in the Uppsala cohort).

Serum samples were drawn in the morning (before 1000 h; 69% of the cohort) or around noon (between 1000 and 1500 h, average 1300 h; 31%). Serum levels of DHEA were lower (–28.7%, $P < 0.001$) in the men with nonmorning samples compared with levels in men with morning samples, but there was no difference in DHEA-S levels (+2.2%, $P = 0.51$). Adjustment for the time of serum sampling (morning sample, yes/no) did not influence the results on the relation between DHEA and mortality end points; hazard ratio (HR) of low DHEA (quartiles 1 *vs.* 2–4) for all-cause death was 1.49 [95% confidence interval (CI) 1.18–1.88] without adjustment and 1.49 (1.18–1.89) with adjustment. Therefore, further analyses were not adjusted for time of serum sampling.

Covariates, serum sex steroids, and mortality data

For description see supplemental data, published on The Endocrine Society's Journals Online web site at <http://jcem.endojournals.org>.

Statistical analysis

We used Cox proportional hazards regression to analyze the associations between serum DHEA, DHEA-S, and mortality outcomes. DHEA and DHEA-S were examined as quartiles based on the entire population. Based on these results, DHEA and DHEA-S were further examined as dichotomous variables comparing quartile 1 to quartiles 2–4. All estimates were adjusted for age and MrOS site. Further adjustments were made for body mass index (BMI) and C-reactive protein (CRP; both log transformed), apolipoprotein (Apo)-B to ApoA1 ratio, and the following dichotomous variables: current smoking, diabetes, hypertension, history of CVD, history of cancer, low testosterone and estradiol (lowest quartile of total serum testosterone and estradiol levels, respectively).

To test whether the associations varied by age, we tested the interaction terms age DHEA (entered as a dichotomous variable; quartile 1 or 2–4) and age DHEA-S in the Cox regression model of all-cause mortality. Additional analyses were performed after stratifying men by age (below or above the median age). Unadjusted Kaplan-Meier survival curves illustrated the association between DHEA-S and all-cause and CVD mortality, and the log-rank test assessed statistical significance.

To further explore the apparent nonlinear relation between DHEA-S and mortality risk, Poisson regression models were used to estimate the hazard function (19). In the Poisson models, spline functions were applied to study the association between serum levels of DHEA-S and the risk of all-cause or CVD death, with age and time included as independent variables. The logarithm of the hazard function was made up of linear pieces at the ends and quadratic functions in the intermediate intervals.

Because of a nonnormal distribution of DHEA and DHEA-S, Spearman rank correlations were used to assess the univariate associations between serum DHEA, DHEA-S, testosterone, and estradiol. The association between age and DHEA(-S) was determined by linear regression. Student's *t* tests were used to test

TABLE 1. Baseline characteristics of the study participants

Variable	All participants (n = 2644)	Within Q1 of DHEA-S (n = 661)	Within Q2–4 of DHEA-S (n = 1983)	P
Age (yr)	75.5 ± 3.2	76.3 ± 3.1	75.2 ± 3.2	<0.001
BMI (kg/m ²)	26.4 ± 3.6	26.7 ± 3.6	26.2 ± 3.6	0.005
ApoB (g/liter)	1.07 ± 0.25	1.06 ± 0.25	1.08 ± 0.24	0.10
ApoA1 (g/liter)	1.53 ± 0.30	1.51 ± 0.31	1.54 ± 0.29	0.050
ApoB/A1	0.72 ± 0.20	0.72 ± 0.21	0.72 ± 0.20	0.85
hsCRP (mg/liter) ^a	2.61 ± 2.37	2.78 ± 2.58	2.56 ± 2.30	0.040
Current smoking (%)	8.5	5.7	9.4	0.003
Diabetes (%)	9.3	13.0	8.0	<0.001
Hypertension (%)	34.9	39.0	33.6	0.013
History of cancer (%; n)	15.8	20.5	14.2	<0.001
History of CVD (%)	26.6	34.5	24.0	<0.001
DHEA (ng/ml)	1.76 ± 1.20	0.90 ± 0.56	2.04 ± 1.22	<0.001
DHEA-S (μg/ml)	0.70 ± 0.46	0.23 ± 0.09	0.85 ± 0.42	<0.001
Testosterone (ng/ml)	4.50 ± 1.87	4.28 ± 2.05	4.57 ± 1.80	0.001
Estradiol (pg/ml)	20.9 ± 7.9	20.3 ± 8.7	21.1 ± 7.7	0.024

Values are given as mean ± sd unless otherwise indicated. hsCRP, High-sensitivity CRP; Q, quartile.

^a Geometric mean ± sd.

the difference in mean DHEA(-S) concentrations between morning/nonmorning samples, survivors/nonsurvivors, and baseline variables in quartile 1 *vs.* 2–4 as well as time to death in participants below or above the median age. Possible differences in the frequencies of prevalent CVD, diabetes, and hypertension between men within quartile 1 *vs.* 2–4 or below/above the median age were tested by χ^2 test. We performed statistical analyses using SPSS for Windows (version 13.0; SPSS, Chicago, IL).

Results

Table 1 shows the baseline characteristics of the study cohort (n = 2644) when their median age was 75.4 yr (range 69–81 yr).

Spearman rank correlation showed a correlation coefficient of 0.73 ($P < 0.001$) between DHEA and DHEA-S. Both DHEA ($r = -0.22$, $P < 0.001$) and DHEA-S ($r = -0.20$, $P < 0.001$) were negatively correlated with age, with a decrease of 0.084 ng/ml and 0.029 μg/ml per year for DHEA and DHEA-S, respectively.

During the mean follow-up interval of 4.5 yr (11 880 person-years), 328 persons (12.4%) died, yielding a mortality rate of 27.6 per 1000 person-years. There was no loss of follow-up with respect to all-cause mortality. Because death certificates were missing for 18 of the men who died during the follow-up, cause of death could be determined for 310 men; 123 (40%) of these deaths were attributed to CVD and 127 (41%) were attributed to cancer.

Age-adjusted Cox proportional hazards regression analyses revealed an inverse association between quartiles of DHEA and DHEA-S and all-cause mortality ($P_{\text{trend}} = 0.002$; Table 2). Prespecified analyses showed that risk of all-cause mortality was increased in men within quartile 1

of DHEA and DHEA-S levels compared with men within the individual quartiles 2, 3, and 4. Compared with men within the pooled quartiles 2–4, men with DHEA and DHEA-S levels within quartile 1 had an increased age-adjusted mortality risk (Table 2). This increased mortality risk remained significant after adjustment for smoking, BMI, diabetes, and history of CVD and cancer. The exclusion of men with diabetes or a history of cancer at baseline showed no major influence on all-cause mortality risk at low DHEA or DHEA-S levels (Table 2). The exclusion of men with CVD resulted in a substantial loss of power in the analysis because 129 of 328 of the deaths (39%) were excluded. After exclusion of men with CVD, low DHEA, but not low DHEA-S, remained significantly associated with risk of all-cause mortality.

In analyses of cause-specific mortality, low levels of DHEA and DHEA-S were associated with death from CVD and IHD (Table 3). There was a nonsignificant trend toward increased cancer mortality at low DHEA(-S) levels (Table 3). The risk of death from noncancer, non-CVD causes was increased at low DHEA-S and tended to be increased at low DHEA levels.

Cumulative survival curves (Fig. 1) illustrated that men in the lowest quartile of DHEA-S levels had higher all-cause (Fig. 1A) and CVD (Fig. 1B) mortality compared with men in quartiles 2–4 (log-rank test $P < 0.001$ for both analyses). To further confirm the apparent threshold association between low DHEA-S and risk of CVD death, we performed a spline curve based on Poisson regression, taking DHEA-S level, age, and follow-up time into account (Fig. 2). The curves confirm that the increased incidence of all-cause and CVD mortality emerges at the lowest DHEA-S levels.

TABLE 2. HRs of DHEA and DHEA-S in quartiles for all-cause mortality

	Deaths (n)	DHEA HR (95% CI) ^a	DHEA-S HR (95% CI) ^a
Q1 vs. individual Q2, Q3, and Q4 ^b	328		
Q1		1.00 (referent)	1.00 (referent)
Q2 vs. Q1		0.69 (0.52–0.93)	0.69 (0.52–0.93)
Q3 vs. Q1		0.66 (0.49–0.90)	0.71 (0.53–0.96)
Q4 vs. Q1		0.60 (0.43–0.84)	0.60 (0.44–0.83)
<i>P</i> _{trend}		0.002	0.002
Q1 vs. pooled Q2–4	328	1.49 (1.18–1.88)	1.47 (1.16–1.85)
Q1 vs. pooled Q2–4 (adjustment for current smoking, BMI, diabetes, and history of cancer and CVD)		1.33 (1.05–1.70)	1.38 (1.09–1.75)
Q1 vs. pooled Q2–4 excluding:			
Men with diabetes	277	1.48 (1.15–1.92)	1.39 (1.07–1.80)
Men with a history of cancer	249	1.40 (1.07–1.84)	1.39 (1.06–1.83)
Men with a history of CVD	199	1.49 (1.09–2.02)	1.32 (0.96–1.81)

^a HRs have been adjusted for age.

^b DHEA quartiles: quartile 1, 0.98 or less; quartile 2, 0.98–1.48; quartile 3, 1.48–2.20; quartile 4, 2.20 ng/ml or greater. DHEA-S quartiles: quartile 1, 0.37 or less; quartile 2, 0.37–0.60; quartile 3, 0.60–0.92; quartile 4, 0.92 μg/ml or greater.

In an attempt to limit the potential influence of prevalent acute/subacute diseases on DHEA(-S) levels, we performed analyses that excluded participants with a follow-up time of 3 yr or less (*i.e.* death within the first 3 yr of follow-up) as a marker for occult disease. The results for CVD and IHD mortality were strengthened with this exclusion, but there was no material change in the results for all-cause mortality (Table 3).

Adjustment for the traditional cardiovascular risk factors (age, smoking, BMI, diabetes, hypertension, and ApoB to ApoA1 ratio) in a multivariate model did not materially change the point estimates for the risk of CVD

TABLE 3. HRs of low levels (quartile 1 vs. pooled quartiles 2–4) of DHEA and DHEA-S for cause-specific mortality

	All men (n = 2644) HR (95% CI) ^a	Excluding the first 3 yr of follow-up (n = 2482) HR (95% CI) ^a
All-cause, n	328	166
DHEA	1.49 (1.18–1.88)	1.49 (1.07–2.06)
DHEA-S	1.47 (1.16–1.85)	1.54 (1.10–2.14)
CVD, n	123	53
DHEA	1.72 (1.19–2.50)	2.18 (1.25–3.80)
DHEA-S	1.61 (1.11–2.34)	2.29 (1.32–3.99)
IHD, n	73	33
DHEA	1.94 (1.20–3.13)	2.41 (1.19–4.90)
DHEA-S	1.75 (1.08–2.84)	2.63 (1.31–5.30)
Cancer, n	127	64
DHEA	1.34 (0.92–1.97)	1.20 (0.69–2.09)
DHEA-S	1.34 (0.91–1.96)	1.29 (0.74–2.25)
Noncancer, non-CVD, n	60	32
DHEA	1.61 (0.93–2.78)	1.50 (0.71–3.18)
DHEA-S	1.80 (1.05–3.10)	1.53 (0.72–3.29)

Reference data from Table 2 are in *italics*.

^a HRs have been adjusted for age.

death at low DHEA(-S), whereas the HR of low levels of DHEA for IHD mortality was somewhat reduced (Table 4). To explore inflammation as a potential mechanism for the increased risk of CVD/IHD death at low DHEA(-S) levels, we adjusted for serum CRP levels in the Cox regression analysis. However, adjustment for CRP had no major impact on the increased risk of CVD or IHD death (Table 4).

Serum levels of DHEA and DHEA-S were weakly correlated with serum levels of total testosterone and estradiol (Spearman rank correlation coefficients: DHEA vs. testosterone, 0.14, *P* < 0.001; DHEA vs. estradiol, 0.09, *P* < 0.001; DHEA-S vs. testosterone, 0.04, *P* = 0.03; DHEA-S vs. estradiol, 0.04, *P* = 0.03). Because we previously found that low levels (lowest quartile) of serum testosterone and estradiol showed an association with all-cause, but not CVD, mortality (18), we adjusted the analyses of DHEA(-S) and mortality for both low testosterone and estradiol levels. However, the association between low DHEA and DHEA-S and all-cause as well as CVD and IHD mortality remained materially unchanged after adjustment for low serum testosterone and estradiol (Table 4).

To study the potential modifying effect of age with the association between low DHEA and DHEA-S and all-cause mortality, we tested the interaction terms age DHEA and age DHEA-S in the Cox regression model. Because both interaction terms were significant (*P* < 0.05), we performed an analysis stratified by median age, 75.4 yr (Table 5). Low DHEA and DHEA-S predicted all-cause mortality and CVD and IHD death only in men less than the median age (Table 5). In an exploratory analysis to understand the age interaction, we performed additional comparisons between the two age groups. The time to death did not differ significantly between younger-old

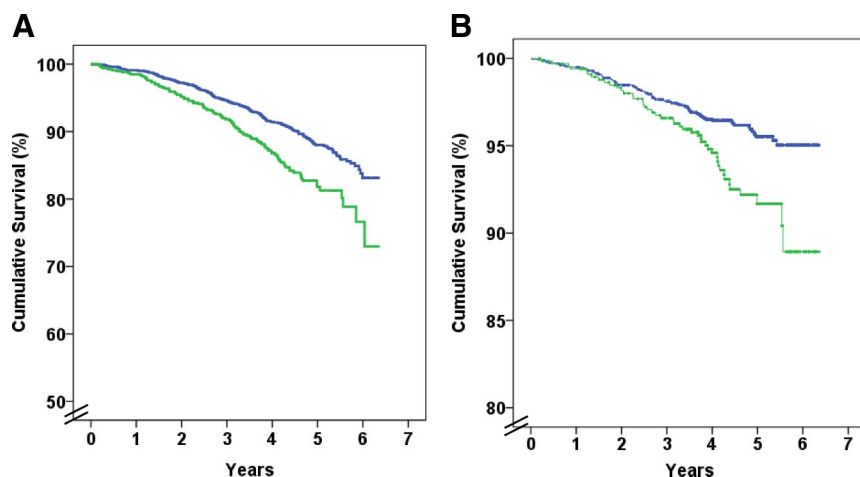


FIG. 1. Kaplan-Meier survival curves for all-cause (A) and CVD (B) mortality by serum DHEA-S levels. Green, Within quartile 1; blue, quartiles 2–4. $P < 0.001$ by log-rank test.

(time to all cause death 3.08 ± 1.38 ; time to CVD death 2.93 ± 1.23 yr) and older-old men (time to all cause death 2.91 ± 1.38 ; time to CVD death 2.63 ± 1.41 yr). Whereas the frequency of diabetes (9.2% in younger-old *vs.* 9.4% in older-old) and hypertension (36.0 *vs.* 33.8%) was similar, there was a 7% absolute difference in baseline prevalent CVD (23.1 *vs.* 30.1%, $P < 0.001$), which was higher in the older age group. There was no significant difference

between DHEA(-S) and death. Among men in the lowest quartile of DHEA-S, the multivariate-adjusted HRs for all-cause, CVD, and IHD death were 1.54 (95% CI 1.21–1.96), 1.61 (95% CI 1.10–2.37) and 1.67 (95% CI 1.02–2.74), respectively. Analyses with DHEA yielded similar results. The association between low DHEA-S and death remained after adjustment for CRP and circulating estradiol and testosterone levels. The mortality risk prediction by low DHEA(-S) was more pronounced among men younger than the median age 75 yr.

To our knowledge the present study is the largest population-based cohort study ($n = 2644$) addressing the association between DHEA status and all-cause as well as CVD/IHD mortality in men. Furthermore, ours is the first mortality study in which both DHEA and DHEA-S were measured using gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. These techniques provide more accurate assessment of sex steroids than immunoassay-based techniques, especially at low hormone levels (20).

Previous data regarding a possible association between low DHEA(-S) and mortality from all causes are conflicting (8–15, 17). Most cohort studies assessing this association are comparatively small (8, 10–12, 14, 15, 17). Barrett-Connor *et al.* (8) reported a strong association between low DHEA-S levels and 12-yr all-cause and CVD mortality in 242 middle-aged/elderly men from

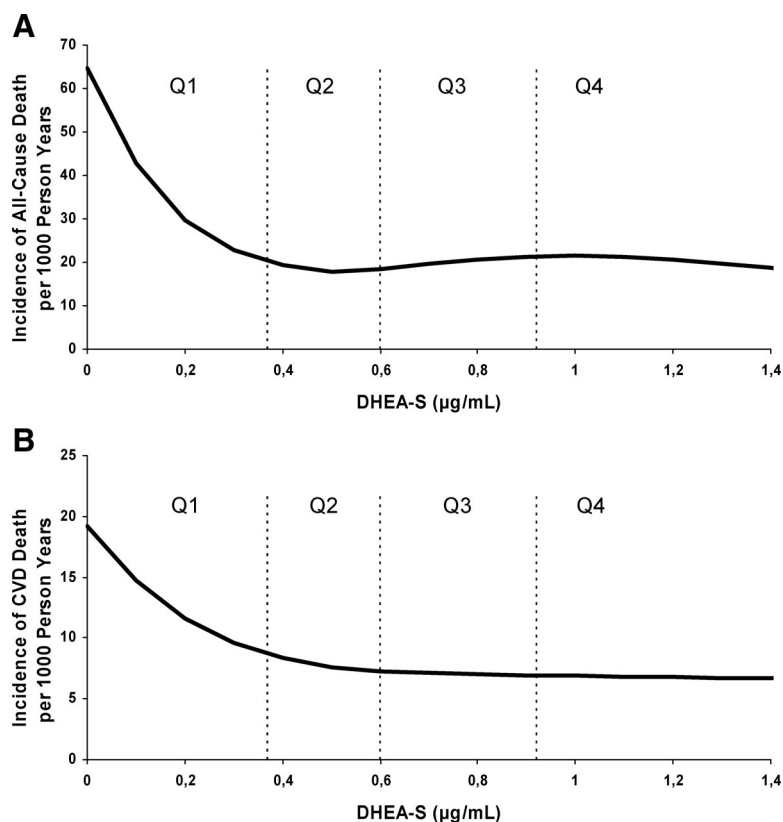


FIG. 2. Yearly incidence of all-cause (A) and CVD (B) death in relation to serum DHEA-S. Poisson regression models were used to determine the relation between serum hormone levels and risk of all-cause and CVD death. For the illustration the age was put equal to 75 yr and the follow-up time to 2 yr. Q, Quartile.

TABLE 4. HRs of low levels (quartile 1 vs. pooled quartiles 2–4) of DHEA and DHEA-S for all-cause, CVD, and IHD mortality with adjustment for covariates

	Adjustment for age, current smoking, BMI, diabetes, hypertension, ApoB to ApoA1 ratio	Adjustment for age, current smoking, BMI, diabetes, hypertension, ApoB to ApoA1 ratio, CRP	Adjustment for age, current smoking, BMI, diabetes, hypertension, ApoB to ApoA1 ratio, low estradiol
	HR (95% CI)	HR (95% CI)	HR (95% CI) ^a
All-cause, n	328		
DHEA	1.49 (1.18–1.88)	1.42 (1.12–1.80)	1.35 (1.06–1.73)
DHEA-S	1.47 (1.16–1.85)	1.50 (1.18–1.90)	1.49 (1.17–1.89)
CVD, n	123		
DHEA	1.72 (1.19–2.50)	1.55 (1.06–2.27)	1.61 (1.09–2.39)
DHEA-S	1.61 (1.11–2.34)	1.59 (1.08–2.33)	1.61 (1.10–2.38)
IHD, n	73		
DHEA	1.94 (1.20–3.13)	1.59 (0.97–2.59)	1.67 (1.01–2.75)
DHEA-S	1.75 (1.08–2.84)	1.65 (1.01–2.71)	1.66 (1.01–2.72)

Reference data from Tables 2 or 3 are in *italics*.

^a Low estradiol and low testosterone entered as quartile 1 vs. 2–4.

TABLE 5. HRs of low levels (quartile 1 vs. pooled quartiles 2–4) of DHEA and DHEA-S for mortality, stratified by age group (below or above the median age 75.4 yr)

	Age < 75.4 yr (n = 1321) HR (95% CI) ^a	Age ≥ 75.4 yr (n = 1323) HR (95% CI) ^a
All-cause, n	124	204
DHEA	2.00 (1.34–2.93)	1.28 (0.96–1.71)
DHEA-S	1.98 (1.34–2.92)	1.26 (0.94–1.69)
CVD, n	39	84
DHEA	2.83 (1.47–5.43)	1.39 (0.90–2.17)
DHEA-S	2.64 (1.37–5.09)	1.30 (0.83–2.04)
IHD, n	25	48
DHEA	3.09 (1.38–6.95)	1.55 (0.86–2.76)
DHEA-S	2.99 (1.32–6.75)	1.35 (0.75–2.46)
Cancer, n	46	81
DHEA	1.24 (0.61–2.51)	1.39 (0.88–2.20)
DHEA-S	1.47 (0.74–2.93)	1.29 (0.81–2.05)
Noncancer, non-CVD, n	33	27
DHEA	2.62 (1.29–5.32)	0.93 (0.40–2.14)
DHEA-S	2.67 (1.30–5.48)	1.20 (0.53–2.68)

^a HRs have been adjusted for age.

the Rancho Bernardo study. However, the finding was not replicated when the whole cohort of men (n = 1029) was studied with a longer (19 yr) follow-up (13). The reason for these discrepant results from the same cohort using the same baseline date and laboratory is unclear (13). In contrast, Trivedi and Khaw (9) reported increased mortality risk at low DHEA-S levels in the Cambridge General Practice Health Study (963 men; mean age 70 yr). Our follow-up time (4.5 yr) was shorter than that of Trivedi and Khaw (7.4 yr), the mean age about 5 yr higher, and the DHEA-S level defining the lowest quartile slightly lower (0.37 μg/ml by liquid chromatography-tandem mass spectrometry in the present study vs. 1.5 μM, corresponding to 0.55 μg/ml, by RIA in the study by Trivedi and Khaw). Nevertheless, the results on all-cause mortality risk are in the same range in this study [quartile 1 of DHEA-S vs. quartiles 2–4; age adjusted HR 1.47 (95% CI 1.16–1.85) in the present study vs. 1.56 (1.14–2.13) in Trivedi *et al.*].

In the present study, low DHEA and DHEA-S predicted increased mortality risk only among older men aged less than the median age of 75 yr but not among men above 75 yr. In line with these results, most previous studies reporting no association between DHEA(-S) and mortality in men have studied the oldest age groups (14, 15, 17), and age heterogeneity is evident in other studies (8, 11). Therefore, differences in baseline age may contribute to the discrepant results of prior studies. Previous data suggest that the dramatic decline in DHEA-S with increasing age is lost among nonsurvivors, in which DHEA-S levels are at a constant, low level over the age span of 50–75 yr (8), and

the present study shows similar results. Given that survivors show an age-related decline in DHEA(-S) levels, this pattern may partly explain why the mortality prediction by DHEA(-S) is depending on age.

Few previous prospective population-based cohort studies addressed DHEA-S as a predictor of CVD and/or IHD mortality in men. Our finding that low DHEA(-S) predicted an increased CVD and IHD mortality risk is similar to the results from the 12-yr follow-up of Rancho Bernardo men (8) but again contrasts the results from the 19-yr follow-up (13). Trivedi and Khaw (9) reported that low DHEA-S predicted death from CVD in elderly men, and the results on CVD mortality risk are in the same range as in the present study [quartile 1 of DHEA-S *vs.* quartiles 2–4: HR 1.61 (1.11–2.34) in the present study *vs.* 1.79 (1.19–2.63) in Trivedi and Khaw (9)]. Data on IHD mortality was not reported in Trivedi and Khaw. In contrast, the Massachusetts Male Aging Study found no significant association between low DHEA(-S) and the 9-yr IHD mortality among 1709 men aged 40–70 yr but did find an association between low DHEA-S and combined fatal and nonfatal IHD events (16). A prospective nested case-control study reported lower DHEA-S among fatal IHD cases (21). Other retrospective case-control studies also reported an association between low DHEA-S and risk of myocardial infarction among men less than age 56 yr (22, 23). Several smaller prospective case-control studies of fatal/nonfatal IHD events (24–26), and a population-based study (27) found no association between DHEA-S levels and IHD risk. Thus, although results of previous studies are contradictory, the present large, but relatively short, study strongly supports an association between DHEA(-S) and CVD/IHD mortality in men.

In the present study, the association between low DHEA(-S) and CVD and IHD death remained significant and was only slightly attenuated after the adjustment for traditional risk factors for CVD. Experimental studies suggest that DHEA may reduce vascular inflammation (6, 7, 28) and oxidative stress (29). In the present study, adjusting the mortality risk estimates for CRP levels did not attenuate the relation between DHEA(-S) and CVD mortality; thus, this analysis does not support increased inflammation (as estimated by CRP levels) as a link between low DHEA levels and CVD death.

A possible mechanism we considered to explain the low DHEA-S association with all-cause and CVD death is its function as an alternate source of androgens and estrogens. We recently reported that low testosterone and estradiol levels, assessed by gas chromatography-mass spectrometry, predict all-cause mortality, but not CVD death, in this cohort of men (18). In the present study, the relation between DHEA(-S) and mortality was not materially

changed after adjustment for low serum testosterone and estradiol levels. Importantly, this does not exclude a pivotal role for androgens and estrogens that are produced locally from the metabolism of DHEA (1).

The large size of the present study provided sufficient power to analyze noncancer, non-CVD mortality risk by DHEA(-S) levels, which has not been reported previously in a prospective population-based study. We found no association with cancer, but noncancer, non-CVD mortality risk was increased in men with low DHEA-S levels.

There are several possible explanations for low DHEA(-S) being associated with CVD death as well as noncancer, non-CVD death in the present study. First, DHEA(-S) has physiological functions that are implicated in CVD as well as noncancer, non-CVD disease defense. For example, DHEA(-S) has been shown to be an efficient activator of peroxisome proliferator-activated receptor- α (2, 28, 29), which may constitute a possible mechanism for its function as a modulator of immune functions (5–7) and oxidative stress (28, 29) as well as atherosclerosis (30). Second, CVD may contribute to deaths classified as noncancer, non-CVD deaths. Several disease processes often contribute to death in the elderly, and death due to coronary heart disease is more frequently misclassified at older ages (31). And third, low DHEA is a general marker of poor health and hence an epiphenomenon of subclinical diseases. The observation that DHEA and DHEA-S levels decrease rapidly during critical illness supports this notion (32). In the present study, the association between low DHEA(-S) and mortality was unchanged after excluding deaths that occurred during the first 3 yr of follow-up, arguing against comorbidity explaining the observed associations. Furthermore, adjustment for clinically manifest (diagnosed) prevalent disease (cancer, CVD, and diabetes) did not alter the association between low DHEA(-S) and mortality, and excluding men with prevalent diseases (cancer, CVD or diabetes) did not materially change the results with a single exception: excluding men with prevalent CVD slightly attenuated the association between low DHEA-S, but not DHEA, and mortality. Importantly, in line with previous findings (8), DHEA did not significantly predict the risk of death due to cancer, which argues against low DHEA being only a passive bystander of age-related comorbidity.

In our study we assessed the serum levels of DHEA and DHEA-S. In line with previous findings that DHEA secretion follows a diurnal rhythm similar to that of cortisol (2), the serum levels of DHEA, but not DHEA-S, were dependent on the time of day blood was drawn. Despite this difference in diurnal variation, analyses with DHEA and DHEA-S yielded similar results in this study in almost every analysis. The associations between DHEA and

DHEA-S with IHD outcomes were also similar in the Massachusetts Male Aging Study (16).

Short-term trials on the effect of DHEA therapy on vascular function in humans have reported both improvement (33, 34) and no effect (35, 36). Similarly, four longer (6 months to 2 yr) trials of DHEA therapy to elderly persons show conflicting results on the effects on body composition and insulin action (37–40). Notably, the latter trials did not select persons with DHEA deficiency. Nair *et al.* (38) and Jankowski *et al.* (39) included subjects (mean age 68–69 yr) with DHEA-S levels less than 1.57 $\mu\text{g/ml}$ (mean 0.6–0.7 $\mu\text{g/ml}$) and 1.40 $\mu\text{g/ml}$ (mean 0.63 $\mu\text{g/ml}$), respectively. According to our results, the increased risk of CVD death in 69- to 81-yr-old men emerges at DHEA-S levels less than 0.37 $\mu\text{g/ml}$. Thus, although available data do not support DHEA supplementation to elderly people, our results may encourage further trials of DHEA therapy to elderly subjects with the lowest DHEA levels.

Study limitations include the use of a single measurement of DHEA and DHEA-S and the use of some non-morning samples given the diurnal variation in serum DHEA levels (2). However, adjustment for the hour of day did not influence the results. Older adults are also being treated with medications, *e.g.* corticosteroids, which might alter mortality risk and/or DHEA levels, which was not examined in this study. In addition, our results are limited to elderly Caucasian men.

In conclusion, in these elderly Swedish men aged 69–81 yr, low serum levels of DHEA and DHEA-S predict death from all causes, CVD, and IHD, independent of traditional risk factors for CVD, CRP, and circulating testosterone and estradiol. The mortality risk prediction by low DHEA(-S) was more pronounced among men younger than the median age 75 yr.

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