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Clinical Research Article

Serum DHEA and Testosterone Levels Associate Inversely With Coronary Artery Calcification in Elderly Men

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Abstract

Context: Epidemiological and preclinical data support cardiovascular, mainly protective, effects of sex steroids in men, but the mechanisms underlying the cardiovascular actions of sex steroids are poorly understood. Vascular calcification parallels the development of atherosclerosis, but is increasingly recognized as a diversified, highly regulated process, which itself may have pathophysiological importance for clinical cardiovascular events.

Objective: To investigate the association between serum sex steroids and coronary artery calcification (CAC) in elderly men.

Methods: We used gas chromatography tandem mass spectrometry to analyze a comprehensive sex steroid profile, including levels of dehydroepiandrosterone (DHEA), androstenedione, estrone, testosterone, estradiol, and dihydrotestosterone, in men from the population-based AGES-Reykjavik study ($n = 1287$, mean 76 years). Further, sex hormone–binding globulin (SHBG) was assayed and bioavailable hormone levels calculated. CAC score was determined by computed tomography. The main outcome measures were cross-sectional associations between dehydroepiandrosterone, androstenedione, estrone, testosterone, dihydrotestosterone, and estradiol and quintiles of CAC.

Results: Serum levels of DHEA, androstenedione, testosterone, dihydrotestosterone, and bioavailable testosterone showed significant inverse associations with CAC, while estrone, estradiol, bioavailable estradiol, and SHBG did not. DHEA, testosterone, and bioavailable testosterone remained associated with CAC after adjustment for traditional cardiovascular risk factors. In addition, our results support partially independent associations between adrenal-derived DHEA and testes-derived testosterone and CAC.

Conclusion: Serum levels of DHEA and testosterone are inversely associated with CAC in elderly men, partially independently from each other. These results raise the question whether androgens from both the adrenals and the testes may contribute to male cardiovascular health.

Key Words: coronary artery calcification, men, atherosclerosis, sex steroids

Abbreviations: BMI, body mass index; CAC, coronary artery calcification; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; GC-MS/MS, gas chromatography tandem mass spectrometry; SHBG, sex hormone–binding globulin.

Sex steroid hormones have crucial roles in reproduction, metabolism, and many other physiological processes. The adrenal androgens dehydroepiandrosterone (DHEA) and androstenedione circulate at high concentrations and are metabolized to active hormones, including testosterone, in peripheral target tissues. Circulating testosterone is mainly derived from the testes in men and may in turn be reduced to the potent androgen dihydrotestosterone (DHT) or aromatized to estradiol in tissues (1).

Epidemiological and preclinical data support cardiovascular, mainly protective, effects of sex steroids in males (1, 2). We have previously shown that higher serum DHEA and testosterone levels are associated with lower risk of cardiovascular events in elderly men (3, 4). In accordance, increased cardiovascular risk has been reported during treatment with androgen deprivation therapy for prostate cancer (5). However, augmented cardiovascular risk has also been reported in studies of testosterone supplementation to elderly

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men, and epidemiological studies report inconsistent findings (6, 7). To date, the mechanisms underlying the cardiovascular actions of sex steroids are poorly understood (1), and increased knowledge in this area is of great clinical relevance.

Vascular calcification, the deposition of hydroxyapatite crystals in the vascular tree, parallels the development of atherosclerosis, but is increasingly recognized as a diversified, highly regulated process, with various triggers besides inflammatory (8). Further, vascular calcification may itself be of pathophysiological importance for clinical cardiovascular events (8). Recent data suggest that coronary artery calcification (CAC) may be a better predictor of future cardiovascular events than traditional cardiovascular risk factors assessed by the Framingham risk index alone (9, 10).

Previous studies addressing a potential association between sex steroids and CAC in men report conflicting results, and most of these have used immunoassays for the analyses of sex steroids, which often have low sensitivity and may be confounded by cross-reactivity (eg, with inflammatory components) (11-13). Cohort studies have reported an inverse association between free/bioavailable testosterone and CAC, but not total testosterone, estradiol, or DHEA levels in men free of cardiovascular disease (14, 15). One study reported an association between low total testosterone levels and CAC in elderly men with stable coronary artery disease (16). In a cross-sectional study of middle-aged men of the Framingham Heart Study, Travison et al found that total and free testosterone was negatively associated with CAC, but not after adjustment for cardiovascular risk factors and prevalent cardiovascular disease (17). Further, a recent study from the Rotterdam study reported no significant associations between sex hormones and CAC (18).

The aim of the present study is to investigate the association between a comprehensive serum sex steroid profile by a highly sensitive gas chromatography tandem mass spectrometry (GC-MS/MS) assay and CAC in a large population-based cohort of elderly men.

Materials and Methods

Study Cohort

This study was performed in the framework of the Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study, which originates from the Reykjavik Study, as described fully elsewhere (19). In summary, 5764 individuals (born January 1, 1907, to December 31, 1935) participated in the baseline examinations of the AGES-Reykjavik Study (2002-2006) (19). AGES men were selected for sex hormone analysis by GC-MS/MS based on availability of extensive genotyping and phenotyping and no history of chemical or surgical castration (n = 1311). Seven men were excluded from further sex steroid analysis due to missing serum tubes (n = 3) or missing or failed GC-MS/MS assay (n = 4). Of the 1304 men with sex hormone data available, computed tomography data for CAC was missing for 17 men, leaving 1287 men for the analysis of sex steroids in relation to CAC. The study was approved by the Icelandic National Bioethics Committee (VSN 00-063-V40) and the intramural institutional review boards of the National Institute on Aging, National Institutes of Health, and the University of Gothenburg, Sweden. All participants gave written informed consent.

Sex Hormone and Sex Hormone–Binding Globulin Analyses

Serum levels of sex steroids were measured by an in-house GC-MS/MS assay, as previously described (20). Briefly, after the addition of isotope-labeled standards, steroids were extracted to chlorobutane, purified on a silica column, and derivatized using pentafluorobenzyl-hydroxylamine hydrochloride followed by pentafluorobenzoyl chloride. Steroids were analyzed in multiple reactions monitoring mode with ammonia as reagent gas using an Agilent 7000 triple quadrupole mass spectrometer equipped with a chemical ionization source. The assay for testosterone was controlled by the Hormone Standardization Project at the Centers for Disease Control and Prevention (Atlanta, GA) (20, 21). The limit of detection for the assay was DHEA 50 pg/mL, androstenedione 4.0 pg/mL, estrone 0.5 pg/mL, testosterone 4.0 pg/mL, estradiol 0.3 pg/mL, and DHT 1.6 pg/mL. All samples passed the internal assay quality check for testosterone, estradiol, and DHT. For the other hormones a few values were omitted at quality check (n = 4 for DHEA, n = 7 for androstendione, n = 3 for estrone). All samples were above the limit of detection for androstendione, estrone, testosterone, estradiol, and DHT. Three samples were below the limit of detection for DHEA; these were given the value of the detection limit in the database.

Sex hormone–binding globulin (SHBG) was assessed by a chemiluminescence immunoassay (Access Immunoassay systems, A48617, Beckman Coulter; RRID: AB_2893035). CV of the assay was 5%. Limit of detection for SHBG was 0.3 nmol/L; all samples were above the limit of detection of the assay. Bioavailable hormones were calculated according to the method of Södergård et al. (22).

Quantification of CAC

Images for calcium scoring were acquired using a Siemens Somatom Sensation 4 multidetector computed tomography scanner (Siemens Medical Solutions, Erlangen, Germany) with prospective electrocardiogram triggering. The electrocardiogram triggering was set at 50% of the cardiac R-R interval. The entire heart was scanned sequentially in the cranio-caudal direction during suspended inspiration (standard scan setting: slice thickness, 2.5 mm; tube voltage, 140 kV, tube current time product, 50 mAs; and scan time 0.361 seconds). Study participants weighing more than 110 kg underwent computed tomography with a tube current setting that was 25% higher than the standard scan setting. The images were reconstructed into a display field of view of 350 mm to include a calibration phantom (Image Analysis, Columbia, KY, USA) that was positioned under the thorax of each subject. The phantom contained calibration cells of 0, 50, 100, 200 mg/cm^3 equivalent concentration of calcium hydroxyapatite. Calcium in the coronary arteries was quantified using the Agatston method (23) by 4 image analysts who had received appropriate training. Phantom-adjusted CAC was expressed as a sum score for all 4 coronary arteries. Interobserver and intraobserver variability assessment showed high reliability of the calcium scoring. Interobserver variability based on the reanalysis of randomly selected 365 scans from the core study population by an expert observer showed an average correlation coefficient of 0.99. Intraobserver variability based on reanalysis of 45 scans by each of the 4 observers resulted in an average correlation coefficient of 0.99. The CAC analysis technique used in this study together with information of its reliability is described in more detail elsewhere (24, 25).

Covariates

Information about smoking habits, diseases and medications was gathered from a questionnaire. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured twice in a supine position using a wall model mercury sphygmomanometer (Erkameter, Erka, Copiague, NY) after a 5-minute rest; hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, self-report of hypertension, or by the use of antihypertensive medications. Diabetes mellitus was defined as a fasting plasma glucose >126 mg/dL (>7.0 mmol/L) or self-reported history of diabetes or the use of insulin or oral glucose-lowering drugs. Prevalent coronary heart disease was defined as a history of coronary events (myocardial infarction diagnosed with electrocardiogram at entry into the study or a history of either myocardial infarctions, coronary artery bypass graft, or percutaneous coronary intervention confirmed by hospital records).

Statistical Analysis

Before statistical analysis, high sex steroid and SHBG levels (at ≥+5 SD; n = 3 for DHEA, n = 8 for androstenedione, n = 1 for estrone, n = 1 for testosterone, n = 5 for estradiol, n = 1 for DHT, n = 4 for SHBG, n = 1 for bioavailable testosterone, n = 3 for bioavailable estradiol) were given a value of the mean value plus 5 SD in the database. Due to skewed distributions, DHEA, androstenedione, and SHBG were $\log_{10}$-transformed before statistical analyses. A correlation matrix of sex steroids and age was analyzed using Pearson correlation. As CAC showed a skewed distribution even after log-transformations, we studied the data across quintiles of CAC score. The associations between covariates and hormones and CAC quintiles were analyzed in unadjusted linear regression models. Hormones were entered as standardized values (Z-score) in multivariate linear regression models including adjustments for age, BMI, current smoking, hypertension (yes/no) and diabetes (yes/no), total cholesterol, and high-density lipoprotein cholesterol ($\log_{10}$-transformed). In sensitivity analyses, hormone levels vs individual CAC score values were also analyzed in an age-adjusted median regression (ie, quantile regression fitted to model median values of CAC score). To address possible nonlinearity in the association between DHEA and testosterone levels and calcification quintiles, hormone levels were added as quadratic term in the age-adjusted linear regression analyses. The interaction term DHEA × testosterone was further studied in a linear regression model including age, DHEA, and testosterone. We divided subjects into 4 groups according to DHEA and testosterone status (above/below median); across these groups we tested differences in CAC using the Kruskal–Wallis test and determined mean CAC quintile in an age-adjusted analysis of covariance. All statistical analyses were performed using SPSS for Windows (version 28, SPSS, Chicago, IL).

Results

The characteristics, including CAC score and sex steroid levels, of the 1287 men from AGES are shown in Table 1. In this elderly population, only a minor part had an Agatston score of 0 in the coronary arteries (n = 45/1287, corresponding to 3.5%). As CAC showed a skewed distribution even after log-transformations, we studied the data across quintiles of CAC score (described in Table 1).

A correlation matrix for sex hormone levels showed that many are highly intercorrelated (Table 2). There were particularly strong associations between DHEA and androstenedione, which are both of adrenal origin. Further, there was a strong association between estradiol and estrone, which is 1 of 2 precursors of estradiol. Testosterone associated strongly with its main carrier protein SHBG as well as its metabolites estradiol and DHT. Further, levels of DHEA, androstenedione and testosterone associated inversely with age, while SHBG associated positively with age.

When analyzing the associations between sex steroids and CAC quintiles in an age-adjusted linear regression model, levels of adrenal-derived DHEA and androstenedione as well as testes-derived testosterone and its metabolite DHT associated inversely with CAC (Table 3). By contrast, there were no statistically significant associations between estrone, estradiol, or SHBG and CAC. We further analyzed the associations between bioavailable testosterone and estradiol and CAC; bioavailable testosterone, but not estradiol, was inversely associated with CAC (Table 3).

After excluding men with prevalent coronary heart disease (n = 441), the age-adjusted association between hormone levels CAC quintile in linear regression models remained significant for DHEA (β −0.102, 95% CI −0.194; −0.011, *P* = .029), testosterone (β −0.112, 95% CI −0.202; −0.023, *P* = .014), and bioavailable testosterone (β −0.146, 95% CI −0.235; −0.056, *P* = .001). To further test the robustness of our findings, we analyzed data using age-adjusted median regression, namely, quantile regression fitted to model median values of CAC score. This analysis also showed a statistically significant association between DHEA (coefficient, ie, difference in median CAC score per SD increase in hormone level, −78.2, *P* = .018), testosterone (−101.3, *P* = .001), and bioavailable testosterone (−107.3, *P* = .001), but not the other hormones, and CAC.

We next evaluated the independent predictors of CACs in multivariate linear regression models including age, smoking, BMI, diabetes mellitus, hypertension, and cholesterol levels (Table 3). Comparing the age-adjusted and age and BMI-adjusted estimates, BMI was an important confounder in some of the observed associations. BMI adjustment yielded slightly reduced estimates but still significant associations for DHEA, androstenedione, testosterone, and bioavailable testosterone but not DHT. After multivariate adjustment, DHEA, testosterone, and bioavailable testosterone, but not androstenedione or DHT, were inverse predictors of CAC (Table 3).

To address possible nonlinearity in the association between DHEA and testosterone levels and calcification quintiles, hormone levels were added as a quadratic term in the age-adjusted linear regression analyses. However, none of these quadratic terms were significantly associated with CAC. To look for potential interaction between DHEA and testosterone, we also tested the interaction term DHEA × testosterone in a CAC regression model including age, DHEA, and testosterone; the result (*P* = .51) supported no significant interaction effect between the 2.

We next entered DHEA and testosterone levels into the same age-adjusted linear regression models. Although estimates were slightly attenuated (DHEA β −0.121, 95% CI

Table 1. Characteristics of the study participants

Variable	All (n = 1287)	Per CAC quintile					*P* (trend across Q)
		Q1 (n = 256)	Q2 (n = 258)	Q3 (n = 258)	Q4 (n = 258)	Q5 (n = 257)	
CAC, median score (range)		30 (0-108)	231 (110-399)	619 (399-875)	1262 (878-1742)	2716 (1747-8673)	—
Age, years	76.4 ± 5.3	75.3 ± 5.1	76.1 ± 5.1	76.3 ± 5.1	76.9 ± 5.4	77.4 ± 5.4	<.001
BMI, kg/m^2	26.9 ± 3.8	26.6 ± 3.9	26.7 ± 3.7	26.7 ± 3.4	27.1 ± 3.8	27.6 ± 3.9	<.001
Current smoking	156 (12.1)	30 (11.7)	24 (9.3)	45 (17.4)	33 (12.8)	24 (9.3)	.021
Hypertension	1020 (79.3)	171 (66.8)	200 (77.5)	207 (80.2)	219 (84.9)	223 (86.8)	<.001
Diabetes mellitus	192 (14.9)	28 (10.9)	38 (14.7)	36 (14.0)	44 (17.1)	46 (17.9)	.85
Total cholesterol, mmol/L	5.18 ± 1.08	5.41 ± 0.96	5.46 ± 1.01	5.20 ± 1.10	5.04 ± 1.10	4.78 ± 1.07	<.001
HDL cholesterol, mmol/L	1.40 ± 0.39	1.42 ± 0.39	1.41 ± 0.40	1.37 ± 0.39	1.40 ± 0.41	1.39 ± 0.38	.41
Statin use	383 (29.8)	29 (11.3)	33 (12.8)	79 (30.6)	93 (36.0)	149 (58.0)	<.001
Prevalent CHD	441 (34.3)	25 (9.8)	52 (20.2)	79 (30.6)	126 (48.8)	159 (61.9)	<.001
DHEA, pg/mL	1412 ± 958	1596 ± 975	1463 ± 1039	1423 ± 987	1370 ± 910	1208 ± 827	<.001
A-dione, pg/mL	960 ± 670	1050 ± 789	995 ± 699	887 ± 518	963 ± 641	901 ± 663	.001
Estrone, pg/mL	29.8 ± 11.1	30.0 ± 9.6	29.7 ± 10.2	29.9 ± 11.0	29.8 ± 12.2	29.7 ± 12.1	.85
T, pg/mL	4190 ± 1707	4500 ± 1723	4360 ± 1712	4086 ± 1561	4124 ± 1775	3883 ± 1701	<.001
Estradiol, pg/mL	22.2 ± 7.8	22.6 ± 6.5	22.4 ± 7.5	22.1 ± 7.7	21.9 ± 8.6	21.9 ± 8.4	.22
DHT, pg/mL	386 ± 200	400 ± 196	398 ± 196	390 ± 194	390 ± 217	350 ± 192	.007
SHBG, nmol/L	53.1 ± 23.3	52.8 ± 22.5	53.5 ± 23.2	50.9 ± 22.6	55.6 ± 23.3	52.7 ± 24.6	.99
Bio-T, pg/mL	3134 ± 1308	3410 ± 1317	3264 ± 1297	3077 ± 1216	3040 ± 1337	2881 ± 1316	<.001
Bio-estradiol, pg/mL	18.9 ± 6.9	19.5 ± 5.8	19.2 ± 6.6	18.9 ± 6.8	18.6 ± 7.7	18.6 ± 7.4	.083

Data are given as mean ± SD or n (%).
Abbreviations: A-dione, androstenedione; Bio, bioavailable; Q, quintile; BMI, body mass index; CHD, coronary heart disease; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; HDL, high-density lipoprotein; T, testosterone; SHBG, sex hormone–binding globulin.

Table 2. Correlation matrix of sex steroids and age

	DHEA	A-dione	Estrone	T	Estradiol	DHT	SHBG	Age
DHEA	—							
A-dione	0.661**	—						
Estrone	0.360**	0.336**	—					
T	0.294**	0.365**	0.341**	—				
Estradiol	0.187**	0.291**	0.639**	0.587**	—			
DHT	0.260**	0.263**	0.253**	0.746**	0.436**	—		
SHBG	0.044	0.114**	0.105**	0.557**	0.205**	0.471**	—	
Age	−0.245**	−0.112**	0.006	−0.070*	−0.024	−0.032	0.219**	—

Analysis by Pearson correlation, correlation coefficients are shown.
Abbreviations: A-dione, androstenedione; DHEA, dehydroepiandrosterone; T, testosterone; DHT, dihydrotestosterone; SHBG, sex hormone-binding globulin.
*P < .05; **P < .001.

−0.203; −0.039, *P* = .004 and testosterone β −0.127, 95% CI −0.207; −0.047, *P* = .002), the results support independent associations for DHEA and testosterone with CAC. We further created 4 groups determined by "high" (above median) or "low" (below median) levels of DHEA and testosterone, respectively (illustrated in Fig. 1A). An age-adjusted analysis of covariance showed that the category with low levels of both DHEA and testosterone showed the highest CAC quintile (Fig. 1B).

Discussion

In the present study, we have addressed the association between sex steroid levels and CAC among elderly men in a large population-based cohort, the AGES-Reykjavik study. Our main finding was that serum levels of DHEA, androstenedione, testosterone, dihydrotestosterone, and bioavailable testosterone showed inverse associations with CAC, while estrone, estradiol, bioavailable estradiol, and SHBG showed

Table 3. Age-adjusted and multivariate associations between sex hormones and CAC

	Age-adjusted model		Age and BMI-adjusted model		Multivariate model	
	β* (95% CI)	*P*	β* (95% CI)	*P*	β* (95% CI)	*P*
DHEA, per SD	−0.158 (−0.237; −0.080)	<.001	−0.135 (−0.214; −0.055)	<.001	−0.107 (−0.184; −0.029)	.007
A-dione, per SD	−0.107 (−0.184; −0.030)	.007	−0.085 (−0.162; −0.008)	.031	−0.068 (−0.144; 0.007)	.076
Estrone, per SD	−0.008 (−0.085; 0.068)	.83	−0.003 (−0.079; 0.073)	.94	−0.003 (−0.078; 0.071)	.93
T, per SD	−0.159 (−0.235; −0.083)	<.001	−0.118 (−0.198; −0.037)	.004	−0.090 (−0.170; −0.010)	.027
Estradiol, per SD	−0.043 (−0.120; 0.033)	.27	−0.047 (−0.123; 0.029)	.22	−0.057 (−0.131; 0.017)	.13
DHT, per SD	−0.102 (−0.178; −0.025)	.009	−0.054 (−0.135; 0.026)	.19	−0.024 (−0.103; 0.055)	.55
SHBG, per SD	−0.043 (−0.122; 0.036)	.29	0.014 (−0.069; 0.097)	.74	0.018 (−0.066; 0.101)	.68
Bio-T, per SD	−0.173 (−0.250; −0.096)	<.001	−0.137 (−0.216; −0.057)	<.001	−0.106 (−0.185; −0.027)	.009
Bio-estradiol, per SD	−0.055 (−0.131; 0.022)	.16	−0.061 (−0.138; 0.015)	.115	−0.067 (−0.141; 0.007)	.075

Linear regression over CAC quintiles.
Multivariate model: Adjusted for age, current smoking, BMI, diabetes mellitus, hypertension, total cholesterol, HDL cholesterol.
Abbreviations: A-dione, androstenedione; Bio, bioavailable; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; SHBG, sex hormone–binding globulin; T, testosterone.
*β coefficient = CAC quintile step per SD increase in hormone level.

no association. DHEA, testosterone, and bioavailable testosterone remained associated with CAC after adjustment for traditional cardiovascular risk factors. In addition, our results support partially independent associations for adrenal-derived DHEA and testes-derived testosterone with CAC.

Immunoassays of sex steroids have been questioned for their limited accuracy and specificity, especially at lower hormone concentrations (11-13), and with the development of mass spectrometry-based assays the demand on more accurate assessment of sex hormones has increased (26). Among the few previous studies addressing the association between sex steroid levels and CAC in men (14-18), only 2 studies used a high-performance sex steroid assay for measurements limited to testosterone (18) or testosterone, estradiol, and estrone (17). In the large (1654 men) population-based Framingham cohort, Travison et al found an inverse cross-sectional association between total testosterone levels and CAC, but the association became nonsignificant after adjustment for cardiovascular risk factors and prevalent cardiovascular disease (17). Further, Travison et al reported a tendency toward a positive association between estrone and estradiol and CAC; in the present study, the trend was the opposite. In the Rotterdam study, Aribas et al found no significant association between testosterone (or other sex steroids) and CAC burden in 719 middle-aged men (18). In the Multi-Ethnic Study of Atherosclerosis (MESA), Khazai et al found an association between free testosterone, but not total testosterone or estradiol levels determined by radioimmunoassay and presence vs absence of CAC in 3164 men (15). Compared with AGES-Reykjavik (mean age 76 years), men in the Framingham and MESA cohorts were younger (mean age 49 and 62 years, respectively). Further, as many as 54% and 39% had CAC score of 0 vs 3.5% in our study, which also may account for the somewhat discrepant findings. In depth comparisons between studies are also hindered by differences in statistical approach to the CAC variable, the selection of which may be important for reported associations (25). In the present study, the results from linear regression over CAC quantiles was not materially different from results obtained by median regression.

We also found a significant inverse association between DHEA levels and CAC, which has not been reported previously. In comparison, the Framingham study reported no results for adrenal androgens in relation to CAC (17), and the MESA study found no association between CAC presence in men and DHEA levels assessed by radioimmunoassay (15). Further, our data suggest that the DHEA and testosterone associations with CAC are at least partially independent of each other, supporting independent contributions of androgens derived from the adrenals and testes, respectively.

Several studies have shown that CAC is highly associated with future cardiovascular risk and that inclusion of CAC in risk estimate scores improves prediction of risk (9, 10). It remains unclear whether this is solely explained by the fact that calcification occurs in atherosclerotic plaques and thereby may serve as a proxy of atherosclerotic burden or if calcification in itself may affect cardiovascular risk, which has been suggested (8). We have previously shown that lower DHEA and testosterone levels were associated with higher risk of cardiovascular events in another population-based cohort of elderly men, the MrOS Sweden cohort (3, 4). Thus, the associations between sex steroids and CAC seem to parallel those with cardiovascular events in elderly men. It is important to note that serum levels of both DHEA and testosterone are decreased in systemic diseases (27) and therefore may be a marker of general health of the participants. In this cross-sectional association study conclusions about causality cannot be drawn, but both DHEA and testosterone exert effects that place them on the list of potentially cardio-protective hormones (2, 27).

Although vascular calcification parallels the development of atherosclerosis, it is conceivable that sex steroids may affect the calcification process via mechanisms other than reduced atherosclerosis. Supporting this notion, preclinical studies have suggested both increased (28, 29) and decreased (30) vascular calcification by testosterone, via androgen receptor-mediated mechanisms. To our knowledge, there are no similar studies for DHEA. Although the vascular calcification process shares features of bone formation at the molecular level, vascular calcification is generally associated with osteoporosis at

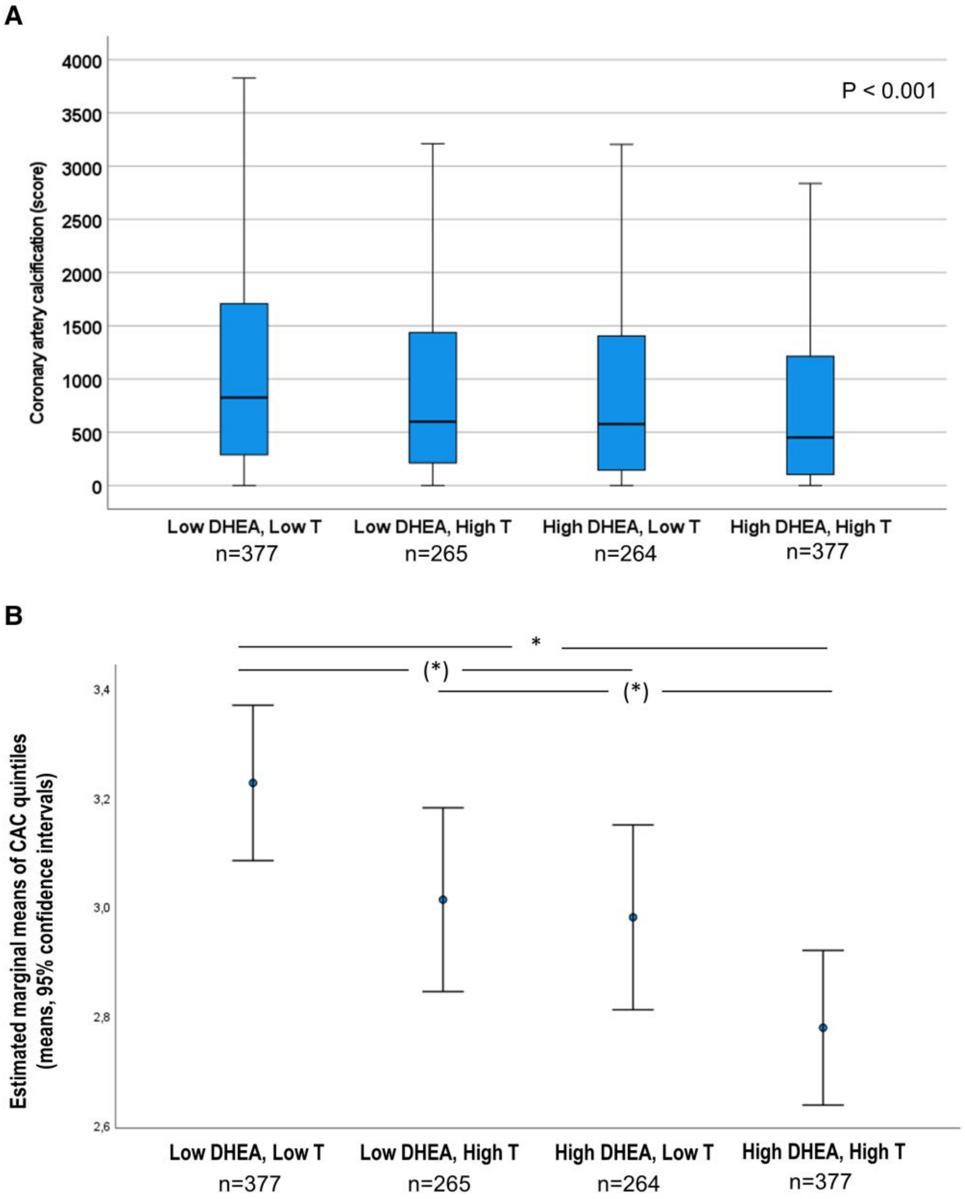

Figure 1. Analyses of CAC across hormone groups. (A) Boxplots of CAC in 4 groups determined by "high" (above median) or "low" (below median) levels of DHEA and testosterone, respectively. Box boundaries represent 25th to 75th percentile, horizontal bars represent median values, whiskers represent upper and lower 5%. *P* value from Kruskal–Wallis test. (B) Age-adjusted estimated marginal means of CAC quintile across hormone groups (analysis of covariance; $P < .001$). (*)$P < .05$ (pairwise comparison without Bonferroni adjustment; >0.05 after adjustment) *$P < .001$ (pairwise comparison with Bonferroni adjustment). DHEA, dehydroepiandrosterone; T, testosterone.

the population level (8). Androgens are important stimulators of bone formation in skeletal bone (31), while our data do not support the hypothesis that they are positive regulators of vascular calcification. Thus, a question that remains unanswered is whether atherosclerosis and calcification in a low-androgen milieu are parallel phenomena or if vascular calcification in addition may be directly regulated by androgens. It has been reported that 1 year of testosterone treatment did not affect CAC, but this study time may be too short to significantly affect the CAC score (32).

The results of the present study lend further support and insight into the relation between sex steroids, in particular adrenal-derived DHEA and testis-derived testosterone, and cardiovascular pathophysiology in men. Future studies should aim to decipher the role of vascular calcification for the cardiovascular risk associated with low androgen levels. As androgen deprivation therapy has been associated with increased cardiovascular risk (5, 7), there is a need for improved understanding in this area. In addition, our findings support the specific concerns that have been raised regarding the cardiovascular safety of the newer treatment modalities for prostate cancer that affect the production of both DHEA and testosterone (33).

Our study has limitations, including all those that are generally associated with a cross-sectional cohort design. Only older men were studied, and our results may not be generalizable to women or men of other ages. Unexpectedly, we found an inverse association between total cholesterol and CAC. Similar findings have been described by others (34) and is likely due to confounding by indication (35) (ie, participants in the higher CAC quintiles have more prevalent coronary heart disease and/or higher cholesterol levels and thereby more statin use leading to a lowering of serum cholesterol). Since CAC may be regarded as a measure of atherosclerosis, which the main mechanism underlying coronary heart disease, which in turn is associated with statin use, we chose not to adjust for prevalent coronary heart disease or statin use in our multivariate analyses to avoid collider bias (35). A recent meta-analysis found no difference between atorvastatin and placebo on the levels of total testosterone in men in randomized controlled trials (36), but the potential role of drug use, including antihypertensive, antidiabetic, and lipid-lowering drugs, for the associations between sex hormones and CAC will require further study. The study also has notable strengths, including the large, well-characterized, and population-based study cohort, nonconfounding by sex, state-of-the-art mass spectrometry-based assessment of sex steroids, and testosterone level validated by the Centers for Disease Control and Prevention, and that all assays were performed in 1 laboratory.

In conclusion, serum levels of DHEA and testosterone are inversely associated with CAC in elderly men, partially independently from each other. These results raise the question whether androgens from both the adrenals and the testes may contribute to male cardiovascular health.

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Disclosures

There are no conflicts of interest.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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