

# Low Levels of Endogenous Androgens Increase the Risk of Atherosclerosis in Elderly Men: The Rotterdam Study

A. ELISABETH HAK, JACQUELINE C. M. WITTEMAN, FRANK H. DE JONG, MIRJAM I. GEERLINGS, ALBERT HOFMAN, AND HUIBERT A. P. POLS

*Departments of Epidemiology & Biostatistics (A.E.H., J.C.M.W., M.I.G., A.H., H.A.P.P.) and Internal Medicine (A.E.H., F.H.d.J., H.A.P.P.), Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands*

**In both men and women, circulating androgen levels decline with advancing age. Until now, results of several small studies on the relationship between endogenous androgen levels and atherosclerosis have been inconsistent.**

In the population-based Rotterdam Study, we investigated the association of levels of dehydroepiandrosterone sulfate (DHEAS) and total and bioavailable testosterone with aortic atherosclerosis among 1,032 nonsmoking men and women aged 55 yr and over. Aortic atherosclerosis was assessed by radiographic detection of calcified deposits in the abdominal aorta, which have been shown to reflect intimal atherosclerosis.

Relative to men with levels of total and bioavailable testosterone in the lowest tertile, men with levels of these hormones in the highest tertile had age-adjusted relative risks of 0.4 [95% confidence interval (CI), 0.2–0.9] and 0.2 (CI, 0.1–0.7), respectively, for the presence of severe aortic atherosclerosis. The corresponding relative risks for women were 3.7 (CI, 1.2–11.6) and 2.3 (CI, 0.7–7.8). Additional adjustment for cardio-

vascular disease risk factors did not materially affect the results in men, whereas in women the associations diluted. Men with levels of total and bioavailable testosterone in subsequent tertiles were also protected against progression of aortic atherosclerosis measured after 6.5 yr (SD  $\pm$  0.5 yr) of follow-up ( $P$  for trend = 0.02). No clear association between levels of DHEAS and presence of severe aortic atherosclerosis was found, either in men or in women. In men, a protective effect of higher levels of DHEAS against progression of aortic atherosclerosis was suggested, but the corresponding test for trend did not reach statistical significance.

In conclusion, we found an independent inverse association between levels of testosterone and aortic atherosclerosis in men. In women, positive associations between levels of testosterone and aortic atherosclerosis were largely due to adverse cardiovascular disease risk factors. (*J Clin Endocrinol Metab* 87: 3632–3639, 2002)

**A**NDROGEN LEVELS DECLINE with advancing age, both in men (1, 2) and women (3). Although it is not known whether this decline in hormonal activity is causally related to physical changes during aging (4), exogenous androgens are considered to be an attractive treatment modality to potentially benefit psychological well-being, body composition, and strength in the elderly (5–7). Dehydroepiandrosterone (DHEA) is being sold in increasing amounts over the counter, several androgen replacement therapy modalities are prescribed for men (5), and its use in women is likely to become more widespread (8). In animal models, treatment with testosterone tended to inhibit the development of atherosclerosis in male rabbits (9), whereas in female monkeys it induced exacerbation of atherosclerosis (10), suggesting gender-specific effects of androgens on cardiovascular disease. In humans, the effects of androgen treatment on cardiovascular disease have not been studied. Endogenous testosterone levels were not found to be related to cardiovascular events in men (11–14) or women (15), whereas studies on endogenous levels of DHEA or DHEA sulfate (DHEAS) and cardiovascular events showed conflicting results (16–18). Results of several studies on endogenous androgen levels and atherosclerosis have been inconsistent (19–25). However, most of these studies were relatively small (19–24).

Abbreviations: BMI, Body mass index; CI, confidence interval; CT, computed tomography; DHEAS, dehydroepiandrosterone sulfate; HDL, high-density lipoprotein; WHR, waist-to-hip ratio.

In the population-based Rotterdam Study, we investigated the association between levels of endogenous androgens and aortic atherosclerosis among a gender-stratified sample of more than 1,000 men and women aged 55 yr and over.

## Subjects and Methods

### *The Rotterdam Study*

The Rotterdam Study is a population-based prospective cohort study designed to assess the occurrence and the determinants of chronic diseases in an aging population (26). The study was approved by the medical ethics committee of the Erasmus Medical Center (Rotterdam, The Netherlands). The cohort includes 3,105 men and 4,878 women aged 55 yr and over (78% of the eligible population) living in a defined district in Rotterdam. Written informed consent was obtained from all participants. Baseline data were collected from 1990 until 1993. The third examination phase took place from 1997 until 1999. Between these examinations, 1,992 persons died, and 35 were lost to follow-up. Fifty-five subjects were not invited for the third examination phase because they moved outside the area, resulting in 5,901 invited subjects. Of the invited subjects, 1,922 men and 2,875 women (81%) participated.

### *Endogenous androgens*

At the baseline examination of the Rotterdam Study, blood samples were drawn by venapuncture from nonfasting subjects at the research center between 0830 and 1600 h. Levels of steroid hormones were measured in plasma. For the collection of plasma, blood was collected in 5-ml tubes containing 0.5 ml sodium citrate solution. All tubes were stored on ice before and after blood sampling. Platelet-free plasma was obtained by two-stage centrifugation, first for 10 min at  $1600 \times g$  at 4 C and then for 30 min at  $7000 \times g$  at 4 C. Platelet-free samples were immediately frozen in liquid nitrogen and transferred to the laboratory. At the laboratory, plasma samples were stored at  $-80$  C until laboratory studies

were performed. For the purpose of the present study, plasma levels of DHEAS, testosterone, and SHBG were estimated in 12 separate batches of samples using coated tube (testosterone) or double antibody RIAs (DHEAS and SHBG), purchased from Diagnostic Systems Laboratories, Inc. (Webster, TX). Because of the relatively small volumes of plasma available, all values reported are single sample estimations. Intra-assay coefficients of variation, determined on the basis of duplicate results of internal quality control pools with three different levels of each analyte, were less than 4% for SHBG, 13% for testosterone, and 15% for DHEAS. Because interassay variations were relatively large (14% SHBG, 19% testosterone, and 24% DHEAS), we multiplied all concentrations within a batch with a factor, which made results for the internal quality control pools comparable. This reduced interassay variations to 3%, 7%, and 10%, respectively, and was considered justified because the relative differences in the results for the high and middle internal quality control pools per batch were comparable, as evidenced by correlations between these results ( $r = 0.91$  for SHBG,  $r = 0.75$  for testosterone, and  $r = 0.58$  for DHEAS; all  $P < 0.05$ ). Assays were performed blind with respect to information on the subject. As a measure of bioavailable testosterone, non-SHBG-bound testosterone was calculated on the basis of hormone, SHBG, and albumin (see below) levels, and respective affinity constants according to the method described by Södergård *et al.* (27) and van den Beld *et al.* (28).

### Aortic atherosclerosis

At baseline and follow-up, lateral radiographic films of the lumbar spine (T12-S1) were made from a fixed distance while the participant was seated. Atherosclerosis was diagnosed off-line by detecting calcified deposits in the abdominal aorta, as described previously (29, 30), by a technician and scored independently of the subjects' exposure status (in the present study, levels of endogenous androgens). Calcification was considered present when linear densities were present in an area parallel and anterior to the lumbar spine (L1-L4). Values for the extent of calcification were scored according to the length of the involved area (<1 cm, 1–2.5 cm, 2.5–5 cm, 5–10 cm, and  $\geq 10$  cm). We considered the first two classes as mild, the third class as moderate, and fourth and fifth classes as severe atherosclerosis.

Progression of aortic atherosclerosis was defined as the occurrence of new calcifications or enlargement of the calcified area present at baseline. Baseline and follow-up films were examined in pairs. The extent of progression was graded (0.5–1 cm, 1–2.5 cm, 2.5–5 cm, and  $\geq 5$  cm), but because of the relatively small numbers available for analysis, we combined severity grades into two groups: progression absent and progression present. No subject showed a decrease in extent of aortic calcification. All films were read by one observer who was aware of the date of the radiographs. Before the scoring, a sample of the films was read by two observers simultaneously so as to reach agreement on the interpretation of the scoring protocol. Previously determined interobserver agreement on progression scoring (absent *vs.* present), based on 758 pairs of lateral radiographic films of the lumbar spine at our department, reached a percentage of agreement of atherosclerotic change of 88, and a  $\kappa$  statistic of 0.74 (29).

The validity of radiographic assessment of aortic atherosclerosis has been studied by comparing results of this method with data obtained at autopsy. Radiographic assessment was shown to be highly specific, and in most cases visible calcification represented advanced intimal atherosclerosis (31). Intimal calcification was also shown to be clearly distinguishable from medial calcification (32). A comparison study involving computed tomography (CT) was performed at our department. In 56 unselected elderly persons, aortic calcifications were independently assessed by radiography and CT. Calcifications were detected on abdominal radiography in 32 subjects. In all but one person, these calcifications were shown to be located in the aorta on the corresponding CT images (30).

Aortic calcification is known to be associated with risk factors for cardiovascular disease (29, 30) and with atherosclerosis at other sites (33), and it predicts cardiovascular morbidity and mortality (34, 35). When aortic calcification (as detected by radiography) was compared with coronary artery calcium (as detected by electron-beam CT) in 457 participants in the Rotterdam Study, aortic calcification was present in 3.9% of participants in the lowest tertile of coronary artery calcium, in 13.7% of those in the middle tertile of coronary artery calcium, and

in 31.5% of those in the highest tertile of coronary artery calcium ( $P$  for trend  $< 0.001$ , adjusted for age and gender).

### Other variables

During a home interview at baseline, a trained research assistant gathered information on current and past health, medication, smoking habits, and age of menopause (self-reported age of last menstruation). Participants were subsequently invited to visit the research center, where intake of alcohol was assessed using a food frequency questionnaire (36). Height, weight, and waist and hip circumferences were measured while each participant was wearing indoor clothing without shoes. Body mass index (BMI, weight divided by height squared) and waist-to-hip ratio (WHR) were computed. Two blood pressure measurements were taken with a random-zero sphygmomanometer after 5 min of rest with the subject in sitting position and averaged. A venipuncture was performed, and nonfasting blood samples were obtained. They were directly put on ice, and serum samples were processed within 30 min, after which they were kept frozen at  $-20$  C. We used an automated enzymatic procedure to determine serum total cholesterol level (37). High-density lipoprotein (HDL) cholesterol was measured similarly, after precipitation of the non-HDL cholesterol fraction. Albumin was measured using a colorimetric method (KONE Diagnostics, Espoo, Finland). As part of the Rotterdam Study, glucose metabolism was studied using a nonfasting oral glucose tolerance test. Previous results from the Rotterdam Study indicate that nonfasting postload insulin levels are similar to fasting postload levels (38), and it is shown that postload insulin provides a good measure of insulin resistance in nondiabetic subjects (39). Therefore, we used postload insulin as a measure of insulin resistance in nondiabetic subjects. Diabetes mellitus was defined as the use of glucose-lowering medication or a random or postload serum glucose level of at least 11.1 mmol/liter according to the World Health Organization criteria (40).

### Population for analysis

We determined levels of steroid hormones in plasma in a gender-stratified random sample of 1432 subjects (667 men and 765 women). In 1252 subjects (610 men and 642 women), data on aortic atherosclerosis were available. To increase power for the current analyses, we additionally sampled plasma from 233 subjects (116 men and 117 women) with moderate to severe aortic atherosclerosis present at baseline. We excluded participants using systemic corticosteroids (16 men and 26 women) or hormone supplements (1 man and 15 women) at time of blood drawing. One woman used both types of medication, leaving 1428 subjects (709 men and 719 women). All women were postmenopausal. To remove residual confounding by current smoking, which influences levels androgens in men (41, 42) and women (43–45), we additionally excluded smoking men ( $n = 205$ ) and women ( $n = 191$ ), leaving 1032 subjects for the current analyses (504 men and 528 women). Due to logistical reasons and insufficient plasma available, data on DHEAS and total testosterone were missing for 56 men and 44 women, and 76 men and 58 women, respectively. Due to missing data on binding protein levels, data on bioavailable testosterone were additionally missing for 121 men and 114 women. The sex and age-specific prevalence of cardiovascular disease risk factors and aortic atherosclerosis in subjects with missing data on hormone levels were comparable with the prevalence of these risk factors in the 1032 subjects available for the current analyses.

### Statistical analysis

We stratified all analyses by sex to study sex-specific associations. Tertiles of endogenous androgen levels were computed in the randomly selected eligible population (*i.e.* without taking the additionally sampled cases with moderate to severe aortic atherosclerosis at baseline into account). Insulin was natural-log transformed to obtain a normal distribution.

First, we computed age-adjusted levels of cardiovascular disease risk factors according to tertiles of levels of androgens by using general linear models. Tests of significance for the coefficients of the ordered variable of tertiles of androgen levels in subsequent linear regression models with

the cardiovascular disease risk factor as dependent variable were considered to be tests for trend.

Second, we used logistic regression models to compute age and multivariate-adjusted odds ratios for severe aortic atherosclerosis according to tertiles of levels of androgens. Odds ratios as retrieved from these logistic analyses are referred to as relative risks. In these analyses, the number of participants with severe aortic atherosclerosis in subsequent tertiles of androgen levels was compared with the number of participants without any aortic atherosclerosis in these tertiles. Analyses were initially adjusted for age by entering age as a continuous variable in the model. In subsequent models, we additionally adjusted for BMI, systolic blood pressure, cholesterol level, HDL cholesterol level, presence of diabetes mellitus (yes or no), smoking (ever or never), and alcohol intake (in four categories: nondrinking, less than one glass, one to two glasses, and more than two glasses per day). After exclusion of diabetic subjects, we additionally adjusted the analyses for insulin. In analyses regarding women, we additionally adjusted for years since menopause and ever-use of hormone replacement therapy (yes or no).

Third, we used logistic regression models to compute age and multivariate-adjusted odds ratios for progression of aortic atherosclerosis during follow-up according to tertiles of androgen level at baseline. These analyses were additionally adjusted for duration of follow-up.

In all multivariate-adjusted models, we used missing value indicators for missing data on categorical covariates (46), whereas for missing data on continuous covariates we imputed the gender-specific mean value of the respective variable as calculated from the study population of 1032 subjects.

We considered two-sided *P* values less than 0.05 to be statistically significant. SPSS 10.0 for Windows (SPSS, Inc., Chicago, IL) was used for all analyses.

## Results

The baseline characteristics of the study population are shown in Table 1. The age of participating men ranged from 55.0–89.4 yr, with a mean of 67.9 yr. In women, age ranged from 55.1–89.0 yr, with a mean of 69.5 yr. Aortic atherosclerosis was absent in 175 men (35%) and 188 women (36%), whereas severe atherosclerosis was present in 47 men and 46 women (9% in both sexes).

Table 2, A and B, shows the age-adjusted levels of cardiovascular disease risk factors according to tertiles of levels of endogenous androgens. In men, higher measures of body weight were associated with lower levels of DHEAS and testosterone, and former smokers were overrepresented in the lower tertiles of levels of testosterone (Table 2A). Higher blood pressure levels tended to be associated with lower levels of testosterone, although tests for trend did not reach statistical significance. In nondiabetic men, levels of postload insulin were inversely associated with levels of testosterone. In women, higher body weight, BMI, and waist circumference, and lower levels of HDL cholesterol were associated with higher levels of testosterone (Table 2B). In nondiabetic women, levels of postload insulin tended to be positively associated with levels of DHEAS and testosterone, whereas diabetes mellitus tended to be more prevalent in women with lower levels of DHEAS and higher levels of testosterone.

**TABLE 1.** Baseline characteristics of the study sample

Characteristic	Men <sup>a</sup> (n = 504)	Women <sup>b</sup> (n = 528)
Age (yr)	67.9 ± 7.3	69.5 ± 7.9
Weight (kg)	79.4 ± 9.9	69.5 ± 10.6
BMI (kg/m <sup>2</sup> )	26.1 ± 2.9	26.7 ± 3.7
Waist circumference (cm)	94.7 ± 9.4	87.6 ± 10.8
WHR (cm/cm)	0.96 ± 0.07	0.87 ± 0.09
Systolic blood pressure (mm Hg)	138.2 ± 20.3	139.5 ± 21.2
Diastolic blood pressure (mm Hg)	74.8 ± 10.7	72.8 ± 11.0
Total cholesterol (mmol/liter)	6.4 ± 1.1	7.0 ± 1.3
HDL cholesterol (mmol/liter)	1.2 ± 0.4	1.5 ± 0.4
Postload insulin (mU/liter) <sup>c</sup>	43.2 (40.1; 46.5)	49.6 (46.2; 53.3)
Time since menopause (yr)		20.5 ± 9.1
Albumin (g/liter)	43.4 ± 2.6	43.1 ± 2.5
SHBG (nmol/liter)	34.7 ± 14.0	43.8 ± 17.8
DHEAS (μmol/liter)	4.2 ± 2.5	2.6 ± 2.0
Total testosterone (nmol/liter)	11.2 ± 3.9	1.4 ± 0.8
Bioavailable testosterone (nmol/liter)	6.8 ± 2.9	0.7 ± 0.4
Diabetes mellitus (%)	8	8
Former smokers (%)	88	34
Alcohol drinkers (%) <sup>d</sup>	91	74
Ever-use of hormone replacement therapy (%)		14
Aortic atherosclerosis (%)		
Mild	32	30
Moderate	24	26
Severe	9	9

Data represent mean ± SD or percentages.

<sup>a</sup> For some men, data were missing on weight and BMI (n = 1), waist circumference and WHR (n = 31), blood pressure (n = 5), HDL cholesterol (n = 2), albumin (n = 126), SHBG (n = 83), DHEAS (n = 56), testosterone (n = 76), bioavailable testosterone (n = 197), diabetes mellitus (n = 14), and alcohol drinking (n = 50).

<sup>b</sup> For some women, data were missing on BMI (n = 1), waist circumference (n = 39), WHR (n = 40), blood pressure (n = 2), total cholesterol (n = 1), HDL cholesterol (n = 3), albumin (n = 113), time since menopause (n = 12), SHBG (n = 65), DHEAS (n = 44), testosterone (n = 58), bioavailable testosterone (n = 172), diabetes mellitus (n = 24), alcohol drinking (n = 62), and ever-use of hormone replacement therapy (n = 12).

<sup>c</sup> Postload insulin was used as a measure of insulin resistance in nondiabetic subjects only [n = 409 men (excluded are men with diabetes mellitus, n = 41; missing data on diabetes mellitus, n = 14; and men in whom insulin levels were missing, n = 40), and n = 420 women (excluded are women with diabetes mellitus, n = 42; missing data on diabetes mellitus, n = 24; and women in whom insulin levels were missing, n = 42)]. Because insulin was skewed, geometric mean values and 95% CI values in parentheses are shown.

<sup>d</sup> <1 glass; 1–2 glasses; and >2 glasses per day.



**TABLE 2A.** Age-adjusted cardiovascular disease risk factors according to tertiles of levels of endogenous androgens in 504 nonsmoking men<sup>a</sup>

Characteristic	DHEAS ( $\mu\text{mol/liter}$ )			Total T (nmol/liter)			P trend
	Tertile 1 $\geq 0.1$ and $\leq 2.6$	Tertile 2 $> 2.6$ and $\leq 4.6$	Tertile 3 $> 4.6$ and $\leq 15.9$	Tertile 1 $\geq 0$ and $\leq 9.8$	Tertile 2 $> 9.8$ and $\leq 12.6$	Tertile 3 $> 12.6$ and $\leq 36.8$	
Age (yr)	70.6 $\pm$ 0.6	67.3 $\pm$ 0.6	65.8 $\pm$ 0.6	69.9 $\pm$ 0.6	67.9 $\pm$ 0.6	66.3 $\pm$ 0.6	<0.001
Weight (kg)	81.2 $\pm$ 0.8	78.9 $\pm$ 0.8	78.1 $\pm$ 0.8	81.2 $\pm$ 0.9	79.5 $\pm$ 0.8	77.8 $\pm$ 0.8	0.01
BMI (kg/m <sup>2</sup> )	26.6 $\pm$ 0.2	26.1 $\pm$ 0.2	25.6 $\pm$ 0.2	26.5 $\pm$ 0.2	26.3 $\pm$ 0.2	25.6 $\pm$ 0.2	0.004
Waist circumference (cm)	95.9 $\pm$ 0.8	94.8 $\pm$ 0.8	93.1 $\pm$ 0.8	97.1 $\pm$ 0.8	94.5 $\pm$ 0.8	92.6 $\pm$ 0.8	0.014
WHR (cm/cm)	0.96 $\pm$ 0.01	0.97 $\pm$ 0.01	0.96 $\pm$ 0.01	0.97 $\pm$ 0.01	0.96 $\pm$ 0.01	0.95 $\pm$ 0.01	0.92
Systolic blood pressure (mm Hg) <sup>b</sup>	137.4 $\pm$ 1.8	139.9 $\pm$ 1.8	136.8 $\pm$ 1.8	140.2 $\pm$ 1.9	137.6 $\pm$ 1.8	137.0 $\pm$ 1.8	0.78
Diastolic blood pressure (mm Hg) <sup>b</sup>	74.8 $\pm$ 1.0	75.2 $\pm$ 0.9	75.0 $\pm$ 0.9	76.6 $\pm$ 1.0	74.4 $\pm$ 0.9	75.2 $\pm$ 0.9	0.90
Total cholesterol (mmol/liter) <sup>b</sup>	6.3 $\pm$ 0.09	6.4 $\pm$ 0.09	6.4 $\pm$ 0.09	6.3 $\pm$ 0.09	6.4 $\pm$ 0.09	6.3 $\pm$ 0.09	0.53
HDL cholesterol (mmol/liter) <sup>b</sup>	1.2 $\pm$ 0.03	1.3 $\pm$ 0.03	1.2 $\pm$ 0.03	1.2 $\pm$ 0.03	1.2 $\pm$ 0.03	1.2 $\pm$ 0.03	0.67
Postload insulin (mU/liter) <sup>c</sup>	47.6 (41.6; 54.4)	40.1 (35.1; 45.9)	43.3 (37.9; 49.5)	48.2 (42.0; 55.3)	42.9 (37.6; 48.9)	38.8 (34.0; 44.3)	0.34
Diabetes mellitus (%)	9	10	6	8	11	6	0.38
Former smokers (%)	91	82	90	93	88	84	0.91
Alcohol drinkers (%)	92	90	93	90	92	93	0.61

Values are mean  $\pm$  SE or percentages. T, Testosterone.

<sup>a</sup> For some men, data were missing on weight and BMI (n = 1), waist circumference and WHR (n = 31), blood pressure (n = 5), HDL cholesterol level (n = 2), diabetes mellitus (n = 14), and alcohol drinking (n = 50).

<sup>b</sup> 80 men using antihypertensive medication and 17 men using serum lipid-lowering agents were excluded for analyses on blood pressure and cholesterol levels, respectively.

<sup>c</sup> Postload insulin was used as a measure of insulin resistance in nondiabetic subjects only [n = 409 men (excluded are men with diabetes mellitus, n = 41; missing data on diabetes mellitus, n = 14; and men in whom insulin levels were missing, n = 40)]. Because insulin was skewed, geometric mean values and 95% CI values in parentheses are shown.

**TABLE 2B.** Age-adjusted cardiovascular disease risk factors according to tertiles of levels of endogenous androgens in 528 nonsmoking women<sup>a</sup>

Characteristic	DHEAS ( $\mu\text{mol/liter}$ )			Total T (nmol/liter)			P trend
	Tertile 1 $\geq 0.1$ and $\leq 1.5$	Tertile 2 $> 1.5$ and $\leq 2.9$	Tertile 3 $> 2.9$ and $\leq 13.6$	Tertile 1 $\geq 0$ and $\leq 1.0$	Tertile 2 $> 1.0$ and $\leq 1.6$	Tertile 3 $> 1.6$ and $\leq 6.9$	
Age (yr)	71.6 $\pm$ 0.6	70.2 $\pm$ 0.6	66.9 $\pm$ 0.6	68.7 $\pm$ 0.6	69.5 $\pm$ 0.6	69.9 $\pm$ 0.6	0.17
Weight (kg)	69.7 $\pm$ 0.8	69.8 $\pm$ 0.9	69.0 $\pm$ 0.8	67.2 $\pm$ 0.8	69.4 $\pm$ 0.8	70.9 $\pm$ 0.8	0.001
BMI (kg/m <sup>2</sup> )	26.6 $\pm$ 0.3	26.8 $\pm$ 0.3	26.7 $\pm$ 0.3	25.9 $\pm$ 0.3	26.6 $\pm$ 0.3	27.2 $\pm$ 0.3	0.001
Waist circumference (cm)	89.2 $\pm$ 0.9	87.3 $\pm$ 0.9	86.1 $\pm$ 0.9	85.8 $\pm$ 0.9	87.0 $\pm$ 0.9	88.7 $\pm$ 0.9	0.01
WHR (cm/cm)	0.88 $\pm$ 0.01	0.86 $\pm$ 0.01	0.86 $\pm$ 0.01	0.86 $\pm$ 0.01	0.88 $\pm$ 0.01	0.87 $\pm$ 0.01	0.02
Systolic blood pressure (mm Hg) <sup>b</sup>	138.0 $\pm$ 1.7	138.3 $\pm$ 1.8	139.6 $\pm$ 1.7	138.5 $\pm$ 1.6	137.1 $\pm$ 1.7	140.0 $\pm$ 1.7	0.51
Diastolic blood pressure (mm Hg) <sup>b</sup>	73.0 $\pm$ 0.9	72.3 $\pm$ 0.9	72.7 $\pm$ 0.9	72.5 $\pm$ 0.9	73.1 $\pm$ 0.9	73.3 $\pm$ 0.9	0.85
Total cholesterol (mmol/liter) <sup>b</sup>	7.0 $\pm$ 0.1	6.9 $\pm$ 0.1	6.9 $\pm$ 0.1	7.0 $\pm$ 0.1	7.0 $\pm$ 0.1	6.9 $\pm$ 0.1	0.57
HDL cholesterol (mmol/liter) <sup>b</sup>	1.5 $\pm$ 0.03	1.4 $\pm$ 0.03	1.5 $\pm$ 0.03	1.5 $\pm$ 0.03	1.4 $\pm$ 0.03	1.4 $\pm$ 0.03	0.91
Postload insulin (mU/liter) <sup>c</sup>	44.2 (38.7; 50.4)	51.3 (44.9; 58.6)	52.5 (46.5; 59.1)	46.4 (40.9; 52.6)	46.3 (40.6; 52.7)	53.7 (47.0; 61.3)	0.07
Diabetes mellitus (%)	10	8	5	7	8	11	0.06
Former smokers (%)	32	34	39	32	34	37	0.16
Alcohol drinkers (%)	75	75	74	76	78	71	0.75
Ever-use of hormone replacement therapy (%)	17	14	13	19	12	13	0.32
Time since menopause (yr)	20.5 $\pm$ 0.4	20.6 $\pm$ 0.4	20.2 $\pm$ 0.4	20.7 $\pm$ 0.4	20.2 $\pm$ 0.4	20.1 $\pm$ 0.4	0.63

Values are mean  $\pm$  SE or percentages. T, Testosterone.

<sup>a</sup> For some women, data were missing on BMI (n = 1), waist circumference (n = 39), WHR (n = 40), blood pressure (n = 2), total cholesterol level (n = 1), HDL cholesterol level (n = 3), time since menopause (n = 12), diabetes mellitus (n = 24), alcohol drinking (n = 62), and ever-use of hormone replacement therapy (n = 12).

<sup>b</sup> 64 women using antihypertensive medication and 13 women using serum lipid-lowering agents were excluded for analyses on blood pressure and cholesterol levels, respectively.

<sup>c</sup> Postload insulin was used as a measure of insulin resistance in nondiabetic subjects only [n = 420 women (excluded are women with diabetes mellitus, n = 42; missing data on diabetes mellitus, n = 24; and women in whom insulin levels were missing, n = 42)]. Because insulin was skewed, geometric mean values and 95% CI values in parentheses are shown.

In Table 3, A and B, the relative risks for severe aortic atherosclerosis according to tertiles of levels of androgens are shown. Levels of DHEAS were not associated with the presence of severe aortic atherosclerosis in men or women (Table 3, A and B). Men with levels of testosterone in the second and third tertile had lower risks of severe aortic atherosclerosis. Multivariate adjustment did not materially change the results (Table 3A). Women with levels of testosterone in the second and third tertile tended to have higher risks of presence of severe aortic atherosclerosis. Multivariate adjustment diluted the associations (Table 3B). Additional adjustment for waist circumference, or adjustment for waist circumference instead of BMI, did not materially change the results in

either men or women, nor did additional adjustment for postload insulin after exclusion of diabetic subjects (data not shown). Exclusion of male or female participants using serum lipid-lowering or antihypertensive medication did not affect the results either (data not shown).

Of the men with complete data on DHEAS, total testosterone, and bioavailable testosterone, 82% participated in the third examination phase, and in 287, 282, and 208 of these men, respectively, follow-up information of aortic atherosclerosis was available. Of the women with complete data on DHEAS, total testosterone, and bioavailable testosterone, 81% participated in the third examination phase, and in 272, 263, and 197 of these women, respectively, follow-up infor-

**TABLE 3A.** Relative risk (RR) for severe aortic atherosclerosis<sup>a</sup> according to tertiles of levels of endogenous androgens in nonsmoking men

	Aortic atherosclerosis		RR (95% CI) <sup>b</sup>	RR (95% CI) <sup>c</sup>
	Severe (n)	None (n)		
<b>DHEAS tertiles</b>				
≥0.1 and ≤2.6 μmol/liter	15	42	1 (ref)	1 (ref)
>2.6 and ≤4.6 μmol/liter	16	56	1.0 (0.4; 2.3)	0.9 (0.4; 2.2)
>4.6 and ≤15.9 μmol/liter	13	58	0.8 (0.3; 2.0)	0.9 (0.3; 2.2)
			<i>P</i> trend = 0.68	<i>P</i> trend = 0.71
<b>Total T tertiles</b>				
≥0 and ≤9.8 nmol/liter	19	38	1 (ref)	1 (ref)
>9.8 and ≤12.6 nmol/liter	14	48	0.7 (0.3; 1.5)	0.7 (0.3; 1.6)
>12.6 and ≤36.8 nmol/liter	9	60	0.4 (0.2; 0.9)	0.4 (0.1; 1.0)
			<i>P</i> trend = 0.03	<i>P</i> trend = 0.04
<b>Bioavailable T tertiles</b>				
≥0 and ≤5.6 nmol/liter	16	24	1 (ref)	1 (ref)
>5.6 and ≤7.5 nmol/liter	8	36	0.4 (0.1; 1.0)	0.3 (0.1; 0.9)
>7.5 and ≤28.7 nmol/liter	5	43	0.2 (0.1; 0.7)	0.2 (0.0; 0.6)
			<i>P</i> trend = 0.006	<i>P</i> trend = 0.004

T, Testosterone; ref, reference category (lowest tertile of endogenous androgen).

<sup>a</sup> No. of men with severe aortic atherosclerosis compared with number of men without aortic atherosclerosis.

<sup>b</sup> RR as retrieved from logistic regression analysis, adjusted for age.

<sup>c</sup> RR as retrieved from logistic regression analysis, adjusted for age, BMI, systolic blood pressure, cholesterol level, HDL cholesterol level, diabetes mellitus (yes/no), smoking (ever/never), and alcohol intake (4 categories).

**TABLE 3B.** Relative risk (RR) for severe aortic atherosclerosis<sup>a</sup> according to tertiles of levels of endogenous androgens in nonsmoking women

	Aortic atherosclerosis		RR (95% CI) <sup>b</sup>	RR (95% CI) <sup>c</sup>
	Severe (n)	None (n)		
<b>DHEAS tertiles</b>				
≥0.1 and ≤1.5 μmol/liter	16	42	1 (ref)	1 (ref)
>1.5 and ≤2.9 μmol/liter	13	61	0.6 (0.3; 1.6)	0.5 (0.2; 1.5)
>2.9 and ≤13.6 μmol/liter	11	67	0.9 (0.3; 2.3)	0.7 (0.2; 2.2)
			<i>P</i> trend = 0.70	<i>P</i> trend = 0.33
<b>Total T tertiles</b>				
≥0 and ≤1.0 nmol/liter	5	57	1 (ref)	1 (ref)
>1.0 and ≤1.6 nmol/liter	18	57	3.0 (0.9; 9.4)	4.4 (1.1; 17.5)
>1.6 and ≤6.9 nmol/liter	20	54	3.7 (1.2; 11.6)	2.8 (0.7; 11.5)
			<i>P</i> trend = 0.03	<i>P</i> trend = 0.19
<b>Bioavailable T tertiles</b>				
≥0 and ≤0.4 nmol/liter	5	38	1 (ref)	1 (ref)
>0.4 and ≤0.8 nmol/liter	13	43	2.1 (0.6; 7.3)	1.8 (0.4; 8.2)
>0.8 and ≤2.9 nmol/liter	14	48	2.3 (0.7; 7.8)	1.0 (0.2; 5.1)
			<i>P</i> trend = 0.21	<i>P</i> trend = 0.84

T, Testosterone; ref, reference range category (lowest tertile of endogenous androgen).

<sup>a</sup> No. of women with severe aortic atherosclerosis compared with number of women without aortic atherosclerosis.

<sup>b</sup> RR as retrieved from logistic regression analysis, adjusted for age.

<sup>c</sup> RR as retrieved from logistic regression analysis, adjusted for age, BMI, systolic blood pressure, cholesterol level, HDL cholesterol level, diabetes mellitus (yes/no), smoking (ever/never), alcohol intake (4 categories), time since menopause, and ever-use of hormone replacement therapy (yes/no).

mation of aortic atherosclerosis was available. Progression of aortic atherosclerosis during a follow-up period of 6.5 yr ( $SD \pm 0.5$  yr) was observed in 76% of men and 73% of women. In Fig. 1, the age-adjusted odds ratios for progression of aortic atherosclerosis according to subsequent tertiles of levels of androgens are shown. Men in the second and third tertile of levels of total and bioavailable testosterone were protected against progression of aortic atherosclerosis (Fig. 1A). The figure suggests a protective effect of higher levels of DHEAS against progression of aortic atherosclerosis in men, but the corresponding test for trend did not reach statistical significance ( $P = 0.35$ ). Multivariate adjustment did not materially affect the results (data not shown). In women, no association between tertiles of levels of androgens and progression of aortic atherosclerosis was found (Fig. 1B).

### Discussion

We found an independent, inverse association between levels of endogenous testosterone and severe aortic atherosclerosis and progression of aortic atherosclerosis in men. In women, higher levels of testosterone tended to be positively associated with severe aortic atherosclerosis and progression of aortic atherosclerosis, although multivariate adjustment diluted the associations. We found no clear association between levels of DHEAS and presence of severe aortic atherosclerosis, either in men or in women. Our findings suggested a protective effect of higher levels of DHEAS against progression of aortic atherosclerosis in men, but the corresponding test for trend did not reach statistical significance.

When interpreting our results, some methodological issues should be taken into account. Due to small volumes of plasma available, we were not able to run the assays in duplicate, and the single sample measurement will have led to less precise estimations of plasma levels. Furthermore, a relatively large proportion of free testosterone measurements, although random, was missing. Although these factors will have reduced the precision of our risk estimates, they will only have led us to underestimate the strength of the associations.

In our study sample, cardiovascular disease risk factors tended to be more adverse in men with lower levels of

testosterone, whereas in women atherogenic changes in cardiovascular risk factors tended to be associated with higher levels of testosterone, which corresponds with previously published data in men (2) and women (47). The positive association between testosterone and cardiovascular disease risk factors in women largely accounted for the positive association between testosterone and aortic atherosclerosis found in women.

Lower levels of testosterone and free testosterone have been described in 55 male subjects with angiographically measured coronary atherosclerosis (21). The same author found in 60 postmenopausal women undergoing diagnostic coronary angiography free testosterone levels to be positively associated with degree of coronary atherosclerosis (22). Results of both described studies (21, 22) are in agreement with our results. In a case-control study conducted within the Edinburgh Artery Study among 83 subjects with peripheral arterial disease and a comparable number of controls, however, no association with testosterone was found in either men or women (23). This discrepancy of results may be attributable to the limited sex-specific power of this study and the fact that peripheral arterial disease may encompass subjects with less severe atherosclerosis than the subjects with severe aortic atherosclerosis in our study. Contrary to our results, a recent cross-sectional study in 101 premenopausal and postmenopausal women found that women in the highest tertiles of testosterone had significantly lower carotid intima-media thickness independent of cardiovascular disease risk factors (24). Similar results were obtained when analyses were restricted to the 48 postmenopausal women (24). The apparent discrepancy between our results and the results of studies in which no association between endogenous testosterone levels and coronary heart disease in men was reported (12–14) may be attributable to the fact that we studied nonsmokers only and to the fact that the aorta might be more vulnerable to the effects of endogenous sex steroids than other arteries. Aortic atherosclerosis has been found to be associated with an up to nine times increased risk of ischemic stroke (48), indicating its importance in relation to cardiovascular disease. Mechanisms possibly involved in the association between aortic atherosclerosis and stroke

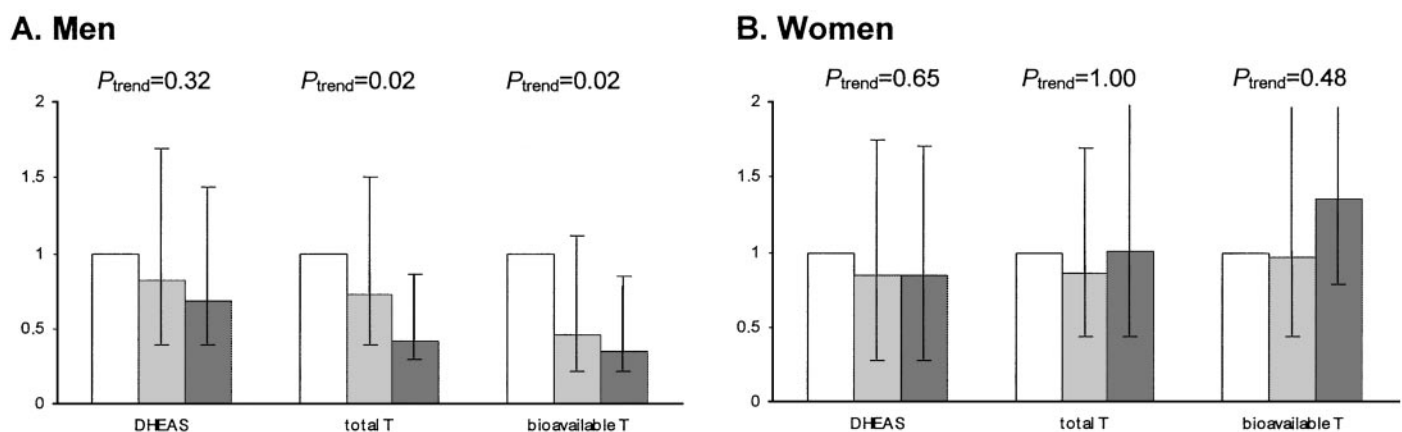


FIG. 1. Age-adjusted odds ratios for any progression of aortic atherosclerosis in nonsmoking men (A) and women (B). White, light gray, and darker gray columns indicate first, second, and third tertiles, respectively, of baseline levels of endogenous androgens. T, Testosterone.

may be pulse pressure or emboli being released from atherosclerotic lesions in the aortic arch.

The mechanisms of the beneficial effect of testosterone on atherosclerosis in males are largely unknown (49). It has been suggested that testosterone may affect atherosclerosis through modulation of classical cardiovascular disease risk factors (49). The fact that multivariate adjustment did not influence the association between testosterone and atherosclerosis in men in our study sample does not support this hypothesis. Negative associations between testosterone and the hemostatic risk factors plasminogen activator inhibitor I (21, 50), fibrinogen (21, 50), and factor VII (51) have been reported in men, indicating that testosterone may affect atherogenesis through a modulation of these factors. As suggested by recent animal experiments, direct beneficial effects of testosterone on plaque development, probably mediated by the vascular androgen receptor, may be involved (52). Another explanation for our results that should be considered, however, is the hypothesis that higher levels of testosterone do not protect against atherosclerosis in men, but are merely a marker of good health (4).

DHEAS is the most abundantly produced adrenal steroid. It is considered to be a weak androgen, mainly contributing to androgenicity by its peripheral conversion to the more potent androgens testosterone and dihydrotestosterone. It has been suggested that DHEAS exerts antiatherogenic effects (53). Nested case-control studies conducted within large cohort studies failed to find an association between levels of DHEAS and the onset of cardiovascular disease in men (16, 17). Results from a prospective cohort study showed that high levels of DHEAS decreased the risk of fatal heart disease in middle-aged men, whereas in women DHEAS offered no protection (18). Similar sex differences were reported with regard to coronary atherosclerosis measured by angiography (19). In both men and women, a negative correlation between levels of DHEAS and pulse wave velocity of the aorta, an indicator of atherosclerosis, was found (20), and in women a negative association between levels of DHEAS and carotid intimal-medial thickness was described (24). Within the prospective population-based Bruneck Study, however, DHEAS was not found to be associated with development and progression of carotid atherosclerosis among 867 subjects during 5 yr of follow-up (25). Although we found no clear association between levels of DHEAS and presence of severe aortic atherosclerosis, our findings suggested a protective effect of higher levels of DHEAS against progression of aortic atherosclerosis in men. The corresponding test for trend, however, did not reach statistical significance. We cannot exclude the possibility that the lack of a significant finding in our study is due to limited sample size. The inconsistency of results on the association between DHEAS and cardiovascular disease indicate that further studies should try to elucidate this issue, particularly given the high frequency of DHEA as a treatment for aging.

In recent years, testosterone replacement strategies have been developed for men (5), and new preparations developed specifically for women are becoming available (8). Many of their aspects, however, remain controversial, and increasing blood hormone levels to those found in 30- to 50-year-old individuals has not yet been uniformly proven to

be safe and of benefit (4). We have to be careful to extrapolate our results regarding the association between endogenous testosterone levels and aortic atherosclerosis to potential effects of therapeutic application of androgens. Dose, duration, the identification of elderly who might benefit most, and possible effects on the process of atherosclerosis of testosterone supplementation remain subjects for study (4).

In conclusion, we found an independent inverse association between levels of testosterone and severe aortic atherosclerosis in men. In women, higher levels of testosterone tended to be positively associated with severe aortic atherosclerosis, which was largely accounted for by more adverse cardiovascular disease risk factors. Whether treatment with testosterone may protect against atherogenesis in men remains to be studied.

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Address all correspondence and requests for reprints to: H. A. P. Pols, Department of Internal Medicine, Room D429, Erasmus Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. E-mail: pols@inw3.azr.nl.

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