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Circulating androgen levels are associated with subclinical atherosclerosis and arterial stiffness in healthy recently menopausal women

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ABSTRACT

Although increasing evidence supports an association between endogenous sex hormones and cardiovascular disease, the results still remain controversial. This study aims to examine the association between endogenous sex hormones and indices of vascular function and structure. Serum follicle-stimulating hormone, luteinizing hormone, estradiol, testosterone, sex hormone–binding globulin, dehydroepiandrosterone sulfate (DHEAS), and Δ4-androstenedione were measured in 120 healthy postmenopausal women aged 41 to 60 years. Possible associations with surrogate markers of subclinical atherosclerosis, arterial stiffness, and endothelial function were investigated. Indices of arterial structure included carotid and femoral intima-media thickness and atheromatous plaques presence. Indices of arterial function included flow-mediated dilation of the brachial artery, carotid-femoral pulse wave velocity (PWV), and augmentation index. Total testosterone and free androgen index (FAI) were the most important predictors of common carotid artery intima-media thickness (β = 0.376 and β = 0.236, $P < .001$ and $P = .014$, respectively). Similarly, FAI was the only significant independent predictor of PWV (β = 0.254, $P = .027$) after adjusting for age, smoking, body mass index, homeostasis model assessment of insulin resistance, and blood lipids. Free estrogen index showed a positive association with PWV, independently of age, smoking, and body mass index, but not of homeostasis model assessment of insulin resistance and blood lipids. Age-adjusted levels of DHEAS exhibited a significant independent negative association with measures of augmentation index. Follicle-stimulating hormone, luteinizing hormone, estradiol, sex hormone–binding globulin, and Δ4-androstenedione were not associated with any of the vascular parameters independently of traditional cardiovascular risk factors. Higher serum

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testosterone and FAI are associated with subclinical atherosclerosis in healthy recently menopausal women. This association is independent of traditional cardiovascular risk factors or insulin resistance. On the contrary, serum DHEAS exhibits a negative association with arterial stiffness.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide for both sexes [1,2]. Women develop CVD typically after the menopausal transition and approximately 10 years later than men. The pattern of symptoms differs with respect to sex; women have more frequently atypical cardiac complaints than men, leading to higher case fatality rate [1,3-5]. Recent results from the Framingham Heart Study report sex differences in the prevalence, lifetime risk, and age of first incidence of stroke [4,5]. Most studies evaluating CVD risk factors have been conducted on men or mixed populations, whereas few studies specifically address postmenopausal women [2].

Established surrogate markers of early CVD such as intima-media thickness (IMT), carotid-femoral pulse wave velocity (PWV) and adjusted augmentation index (AI) can be assessed by noninvasive and easily accessible methods, providing information on the cardiovascular function and structure of an asymptomatic individual and predicting future cardiovascular events [6-8]. Intima-media thickness is an emerging risk factor with reliable reproducibility, which can be used to track the progress of subclinical disease during follow-up examinations [7,9,10]. Furthermore, the criterion standard method to assess arterial stiffness is considered to be PWV, defined as the speed of systolic pulse transmission from the left ventricle to peripheral arteries, being higher in stiff arteries [11,12]. The AI indicates the stiffness of the systemic arterial tree, assessing the interaction between incident and reflected pulse wave [8,11]. Finally, endothelial function can be assessed by flow-mediated dilation of brachial artery (FMD), an index of endothelial nitric oxide (NO) bioavailability, which is estimated by measuring the brachial artery diameter before and after the application of shear stress induced by reactive hyperemia [13,14].

Endogenous sex hormones may affect the cardiovascular risk profile in postmenopausal women [15], indirectly by inducing hemodynamic, metabolic, and immunological changes, as well as directly through steroid receptors on the arterial wall [16-19]. The results, however, remain controversial [20-27]. Furthermore, although several studies have focused on structural changes, there are very limited data concerning the association of endogenous sex hormones and functional cardiovascular indices. To the best of our knowledge, no study has assessed endogenous sex steroids in association with functional indices of arterial stiffness and endothelial NO availability in young, apparently healthy, postmenopausal women.

The present cross-sectional study examines associations between endogenous sex hormone levels and subclinical arterial disease by assessing surrogate markers of vascular function and structure in a sample of apparently healthy recently postmenopausal Greek women.

2. Methods

2.1. Subjects

This cross-sectional study included 120 healthy informed consenting women from the Menopause Clinic of the Aretaieio Hospital, University of Athens, postmenopausal for at least 1 year and with intact ovaries. The menopausal status was defined as follicle-stimulating hormone (FSH) greater than 25 mIU/mL and estradiol (E2) less than 50 pg/mL after 12 consecutive months without menses. Before recruitment, patients underwent the routine evaluation of our clinic, including mammography, Papanicolaou test and transvaginal sonography, thyroid-liver-renal function test, blood coagulation tests, and bone densitometry. Inclusion criteria were a sonographically assessed endometrial thickness of less than or equal to 5 mm, absence of current or previous exposure to hormone therapy or raloxifene and absence of gynecological malignancy, clinically overt CVD, premature ovarian failure, thromboembolism, diabetes mellitus, untreated thyroid dysfunction, and treatment with lipid-lowering or antihypertensive medication. Women with adherence and retention concerns (eg, alcoholism) were not included in the study.

2.2. Protocol study procedures

A detailed medical history was recorded for every subject, using questionnaires regarding demographic and lifestyle parameters, cardiovascular risk, and obstetrical and gynecological history. We assessed blood pressure and anthropometric parameters, calculating the waist to hip ratio (WHR) and the body mass index (BMI). Patients were instructed to abstain from food and smoking for 12 hours. Subsequently, fasting venous blood samples were drawn at 8:30 AM to 9:30 AM for the determination of levels of FSH, luteinizing hormone (LH), E2, testosterone, Δ4-androstenedione (Δ4-A), dehydroepiandrosterone sulfate (DHEAS) and sex hormone–binding globulin (SHBG); and the serum was stored at –80°C until assessment.

Ultrasound evaluations were performed immediately thereafter in one session, in a quiet temperature-controlled room, with patients in the supine position, except for the pulse wave analysis that took place in the sitting position. The same operator, blinded to the medical history of the participants, performed the vascular tests. Lastly, after having rested in the sitting position for 5 minutes, 2 blood pressure measures were obtained from each subject by oscillometry using the automated Omron 705IT device (Omron, Japan). The measures were subsequently averaged for use in the data analysis. Institutional Review Board approval was obtained from the Ethics Committee of Aretaieio Hospital.

2.3. Biochemical and hormone assays

Serum glucose, total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were assessed enzymatically by an autoanalyzer (ARCHITECT-ci8200, Abbott Diagnostics Laboratories, Abbott Park, IL; Abbott 65205, Wiesbaden, Germany). The low-density lipoprotein (LDL) cholesterol was estimated using the Friedewald equation (LDL cholesterol = total cholesterol – triglycerides/5 – HDL cholesterol).

The plasma levels of FSH, LH, and E2 were measured on an Architect i1000 analyzer (Abbott Ireland, Diagnostics Division, Lisnamuck, Longford, Ireland), with an analytical sensitivity of 0.05 mIU/mL, 0.07 mIU/mL, and 10 pg/mL, respectively. The total coefficient of variation (CV%) ranged from 3.2% to 4.6% for FSH, from 2.9% to 4.1% for LH, and from 1.9% to 7.1%, for E2. Δ4-androstenedione was measured with the enzyme-linked immunosorbent assay kit (IBL "Androstendione ELISA"; IBL, Hamburg, Germany). The total CV% ranged from 4.7% to 9.7%, and the analytical sensitivity of the assay was 2.0 ng/dL. Total testosterone was measured with the Abbott Architect i1000 analyzer. The total CV% ranged from 3.1% to 8.0%, and analytical sensitivity was 0.08 ng/mL. Dehydroepiandrosterone sulfate was measured by immunoenzymatic assay (Elecsys 2010 Systems, Roche Diagnostics, Monza, Italy). Within-assay CV% was 6%. Sex hormone–binding globulin concentrations were measured with electrochemiluminescence immunoassay on a Cobas e-411 analyzer (Roche Diagnostics, Mannheim, Germany). The total CV% ranged from 2.6% to 5.6%, and the analytical sensitivity of the assay was 0.35nmol/L. Insulin was measured on an Abbott Architect i1000 analyzer. The total CV% ranged from 1.9% to 5.2%, and the analytical sensitivity was 1 μU/mL. Free estrogen index (FEI) and free androgen index (FAI) were calculated using total E2 and total testosterone, respectively, as well as SHBG values by the following equations: FEI = E2 (picograms per milliliter) · 0.367/SHBG (nanomoles per liter); FAI = testosterone (nanograms per milliliter) · 347 /SHBG (nanomoles per liter). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: fasting insulin (microunits per milliliter) · fasting glucose (millimoles per liter)/22.5.

2.4. Ultrasound measurements

2.4.1. Intima-media thickness

Intima-media thickness was measured in 3 paired segments, of both right and left common carotid artery (CCA), carotid bulb (CB), and internal carotid artery (ICA), from a fixed lateral transducer angle using B-mode ultrasound imaging (14.0-MHz multifrequency linear array probe, Vivid 7 Pro, GE Healthcare, Milwaukee, WI). In each segment, 3 measurements of the maximal IMT in the far wall were averaged; and the average IMT was calculated for each of the 2 carotid arteries. The average value of right and left carotid IMT was defined as *combined IMT*. The femoral IMT was measured in the far wall of a 1-cm-long arterial segment proximal to the femoral bifurcation [7]. *Atherosclerotic plaque* was defined as a clearly identified area of focally increased IMT greater than 1.5 mm [7].

2.4.2. Flow-mediated dilation

The ultrasound analysis was performed by 2 independent observers. Flow-mediated dilation was assessed using a 7.0- to 14.0-Hz multifrequency linear array probe attached to a high-resolution ultrasound machine (Vivid 7 Pro, GE). The right brachial artery of each woman was longitudinally imaged 6 cm above the antecubital fossa in a supinated position of the forearm. A pneumatic cuff was placed around the forearm; and after the initial measurements at resting conditions, the cuff was rapidly inflated to 250 mm Hg for 5 minutes and subsequently deflated. The increase of arterial flow (reactive hyperemia) was monitored for 90 seconds. Flow-mediated dilation was calculated as the percentage of maximal change of lumen diameter between rest and reactive hyperemia. The inter- and intraobserver variability for brachial artery diameter measurements in our laboratory is 0.1 ± 0.12 and 0.08 ± 0.19 mm, respectively.

2.4.3. Pulse wave velocity

Carotid-femoral pulse wave velocity was calculated from measurements of pulse transit time and the distance traveled between 2 recording sites with a validated noninvasive device (Complior, Artech Medical, Pantin, France) that allows online pulse wave recording and automatic calculation of PWV (PWV equals distance [meters] divided by transit time [seconds]). The PWV was measured between the CCA and common femoral artery (CV%: 2.4% for 2 repeated measurements).

2.4.4. Pulse wave analysis

The pulse waveform of the aorta was acquired and analyzed using radial artery tonometry (Sphygmocor System; Atcor Medical, Sydney, Australia). Peripheral pressure waveforms were recorded at the radial artery using a handheld high-fidelity tonometer (Millar Instruments, Houston, TX) and calibrated using arterial pressures measured at the brachial artery. Subsequently, aortic pressure waveforms were then calculated by applying generalized transfer functions as previously described [28]. The *AI*, defined as the ratio of the augmentation of systolic pressure induced by the reflected waves to the aortic pulse pressure, was measured in this method and normalized for a heart rate of 75 beats per minute because of the strong dependence of this index on heart rate [8]. Mean difference ± SD for 2 repeated measurements for AI normalized for the heart rate of 75 beats per minute was –0.2 ± 4.3 and for time to the beginning of the reflected wave was –5.2 ± 15.8.

2.5. Statistical analysis

Statistical analysis was performed by SPSS version 17.0 (SPSS, Chicago, IL). The mean IMT of each artery (both left and right side), the combined IMT, and the presence of atherosclerotic plaque at any of the examined sites were set as major representative outcomes for structural subclinical disease. Pulse wave velocity, AI, and FMD were the main outcomes for functional subclinical disease. Data are expressed as percentage values or absolute numbers (mean ± SD). Logarithmic transformation was used in some cases of skewed data. Means of continuous variables were compared between groups by analysis of variance. Correlations between continuous variables were evaluated using Spearman's correlation coefficient. Adjustments for possible confounding factors were performed by multiple regression analysis. Multivariate models were run separately for each hormone together with

traditional cardiovascular risk factors. Statistical significance was set at the .05 level.

3. Results

Table 1 presents the descriptive statistics of the women participating in the study. Concerning the changes of mean arterial IMT according to hormonal quartiles, mean CCA-IMT decreased along with increasing LH levels (0.69 ± 0.14 vs 0.62 ± 0.11 mm in quartiles Q1 and Q4, respectively; $P = .049$). In addition, mean CCA-IMT increased from the lower to the highest quartile of testosterone (0.61 ± 0.12 vs 0.74 ± 0.14 mm in quartiles Q1 and Q4, respectively; $P = .002$) and FAI (0.58 ± 0.12 vs 0.70 ± 0.12 mm in quartiles Q1 and Q4, respectively; $P = .014$ after adjusting for age, BMI, smoking, HOMA-IR, HDL cholesterol, triglycerides, and systolic blood pressure [SBP]). Concerning atherosclerotic plaques, women with one or more atherosclerotic plaques had significantly higher mean LH compared with women with no plaques (ICA 52.9 ± 25.1 vs 35.8 ± 16.9 mIU/mL, $P = .021$; carotid arteries combined 43.8 ± 22.6 vs 34.8 ± 15.8 mIU/mL, $P = .027$; all arteries 43.9 ± 23.1 vs 34.1 ± 14.7 mIU/mL, $P = .009$). Furthermore, concerning the association between mean levels of vascular function indices according to hormonal quartiles, PWV decreased linearly with increasing quartiles of SHBG (9.24 ± 2.10, 8.73 ± 1.65, 8.63 ± 1.90, and 8.01 ± 1.68 m/s in quartiles Q1, Q2, Q3, and Q4, respectively; $P = .028$), whereas PWV increased linearly with increasing quartiles of FAI (8.00 ± 1.70, 8.56 ± 1.58, 8.81 ± 2.16, and 9.39 ± 2.04 m/s in quartiles Q1, Q2, Q3, and Q4, respectively; $P = .016$). Furthermore, measures of AI decreased linearly with increasing quartiles of DHEAS (34.52% ± 7.47% vs 28.05% ± 8.00% in quartiles Q1 and Q4, respectively; $P = .006$).

Table 2 presents the results of the correlation of hormone values with vascular indices. Mean CCA-IMT measures exhibited a significant positive correlation with levels of testosterone and FAI, as well as a negative significant correlation with levels of LH. None of the remaining IMT measures correlated significantly with levels of sex hormones. Among indices of vascular function, PWV correlated negatively with levels of SHBG and positively with levels of FAI, whereas AI correlated only with levels of DHEAS.

The results of the multiple regression analysis with vascular indices as dependent variables and sex hormones as independent variables are shown in Tables 3 and 4. Free androgen index and total testosterone had the most constant effect on CCA-IMT and on combined-IMT, which remained significant even after adjustment for confounding factors (eg age, BMI, current smoking, HOMA-IR, lipids, SBP). These models predicted mean CCA-IMT values with a statistical power of 0.996 and 0.999, respectively, and an effect size of 0.15 ($\alpha = 0.05$, 2 tailed). The association between CCA-IMT and testosterone remained significant after adjusting for E2 and SHBG ($\beta = 0.367$, $P < .001$) or for FEI ($\beta = 0.372$, $P < .001$). Furthermore, FAI was the most significant predictor of PWV, with a statistical power of 0.999 and an effect size of 0.15 ($\alpha = 0.05$, 2 tailed). The results remained significant after adjusting for WHR instead of BMI as an age-related factor of obesity. Free androgen index predicted significantly levels of PWV ($R^2 = 0.270$, $\beta = 0.254$, $P = .027$) as well as mean CCA-IMT values ($R^2 = 0.241$, $\beta = 0.236$, $P = .014$) in multivariate models that included other confounders (eg, age, WHR, current

Table 1 – Demographic characteristics and sex hormones of the 120 postmenopausal women participating in the study

Variables	Mean	Median	SD	Minimum	Maximum
Demographic characteristics					
Age (y)	53.2	54.0	4.1	41.0	60.0
YSM (y)	4.6	4.0	2.8	1.0	10.0
BMI (kg/m^2)	26.8	25.8	4.7	20.1	45.7
Waist (cm)	86.4	86.0	10.5	62.0	114.0
WHR	0.84	0.85	0.06	0.69	0.99
SBP (mm Hg)	117.7	116.3	14.6	81.0	156.0
DBP (mm Hg)	74.3	73.8	8.0	56.5	93.5
FBG (mg/dL)	91.7	92.0	8.6	69.0	114.0
HOMA-IR	1.59	1.33	0.96	0.44	4.95
Total cholesterol (mg/dL)	231.2	233.0	35.3	145.0	322.0
Triglycerides (mg/dL)	92.6	82.0	43.2	37.0	278.0
HDL cholesterol (mg/dL)	61.7	58.0	16.5	22.0	108.0
LDL cholesterol (mg/dL)	143.2	142.0	33.7	67.0	240.0
Insulin (μU/mL)	6.98	6.10	3.68	2.40	19.50
Hormone levels					
FSH (mIU/mL)	76.3	72.7	31.1	26.0	152.5
LH (mIU/mL)	37.0	33.9	17.6	4.1	97.4
E2 (pg/mL)	17.6	12.5	11.2	5.0	48.8
Testosterone (ng/mL)	0.4	0.3	0.2	0.1	1.6
SHBG (nmol/L)	63.1	60.7	28.4	12.5	118.0
DHEAS (μg/dL)	127.7	115.0	62.3	30.0	292.0
Δ4-A (ng/dL)	161.8	140.0	100.2	18.0	432.0
FEI	0.13	0.10	0.16	0.02	1.00
FAI	2.62	2.12	1.84	0.41	9.57

YSM indicates years since menopause; DBP, diastolic blood pressure; FBG, fasting blood glucose.

Table 2 – Correlation between serum levels of sex hormones and arterial IMT using Spearman correlation coefficient

Spearman correlation	CCA-IMT		CB-IMT		ICA-IMT		FA-IMT		Combined-IMT	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
FSH (mIU/mL)	−0.138	.152	0.032	.735	−0.053	.586	−0.078	.423	−0.055	.571
LH (mIU/mL)	−0.202	**.035**	−0.039	.681	−0.010	.917	0.072	.460	−0.083	.389
E2 (pg/mL)	−0.067	.495	−0.081	.404	−0.080	.413	−0.077	.434	−0.113	.248
TESTO (ng/mL)	0.356	**<.001**	0.051	.622	0.005	.964	−0.112	.282	0.179	.084
SHBG (nmol/L)	−0.183	.073	0.041	.687	−0.123	.231	0.137	.183	−0.063	.543
Δ4-A (ng/dL)	−0.040	.719	0.169	.127	0.016	.888	−0.046	.679	0.099	.379
DHEAS (μg/dL)	0.042	.686	0.004	.972	−0.149	.153	−0.008	.940	−0.008	.943
FEI	0.012	.906	−0.198	.055	−0.052	.621	0.045	.669	−0.111	.293
FAI	0.353	**.001**	−0.023	.829	0.036	.737	0.021	.845	0.112	.293

	FMD		PWV		AI	
	r	P value	r	P value	r	P value
FSH (mIU/mL)	0.089	.355	−0.144	.132	0.006	.948
LH (mIU/mL)	0.068	.475	−0.090	.346	−0.061	.539
E2 (pg/mL)	−0.063	.517	0.026	.787	0.011	.912
TESTO (ng/mL)	−0.087	.401	0.090	.387	−0.046	.674
SHBG (nmol/L)	0.025	.807	−0.217	**.032**	0.000	.995
Δ4-A (ng/dL)	0.184	.098	−0.154	.167	−0.057	.627
DHEAS (μg/dL)	0.153	.140	0.146	.163	−0.247	**.022**
FEI	−0.007	.948	0.133	.200	0.059	.590
FAI	−0.093	.379	0.244	**.019**	0.003	.975

Bold indicates statistical significance: $P < .05$. TESTO indicates testosterone; FA, femoral artery.

smoking, HOMA-IR, and lipids). The inclusion of E2 to the model did not modify the results significantly (CCA-IMT β = 0.223, P = .02; PWV β = 0.189, P = .048). The association between levels of FAI and PWV lost statistical significance after including SBP as a covariate. However, levels of FAI exhibited a significant independent correlation with values of SBP (r = 0.244, P = .019), indicating a possible mediating effect of the association between FAI and PWV. Levels of FEI had a significant effect on PWV; the inclusion of insulin in the model, however, attenuated the effect of FEI. Finally, DHEAS was the only hormone exhibiting a significant effect on measures of AI, even in the multivariate model (R^2 = 0.103, β = −0.267, P = .029) that included other confounders (eg, age, BMI, current smoking, HOMA-IR, lipids, and SBP).

4. Discussion

The present study demonstrates a significant association of circulating androgens with indices of both vascular function and structure in a sample of young, apparently healthy, postmenopausal women. Serum FAI was the most constant predictor of arterial IMT, independently of age, BMI, lipids, blood pressure, and insulin resistance. Furthermore, FAI exhibited an independent association with arterial PWV possibly mediated through SBP. Finally, serum DHEAS predicted significantly arterial stiffness, independently of age, BMI, smoking, lipids, SBP, and insulin resistance.

Accumulating evidence suggests that higher androgenicity in postmenopausal women is associated with an adverse CVD risk factor profile [17-19,29,30] and possibly accelerated subclinical arterial disease [31]. In a recent analysis of the Estrogen Prevention of Atherosclerosis Trial population, the progression rate of carotid IMT was inhibited by estrogen therapy only in women with progressively decreasing testosterone levels [24]. The Multi-Ethnic Study of Atherosclerosis, evaluating 1947 postmenopausal women, found that testosterone levels associated positively with carotid IMT, independently of traditional cardiovascular risk factors [21]. In a subanalysis of the Multi-Ethnic Study of Atherosclerosis population confined to postmenopausal women with abdominal aortic calcifications, SHBG was inversely associated with the presence and extent of abdominal aortic calcification, independently of nonlipid cardiovascular risk factors [22]. In addition, a substudy of the Atherosclerosis Risk in Communities cohort, including only women not on hormone therapy, reported a significant inverse association between SHBG levels and carotid atherosclerosis [25], a finding also reported for young premenopausal women [32]. Beyond arterial atherosclerosis, the progressively increasing androgen-estrogen ratio in women after the menopausal estrogen loss has been associated with the pathogenesis of postmenopausal hypertension [33,34]. In our study, FAI exhibited a strong correlation with SBP. Being a major determinant of arterial stiffness [12], blood pressure could mediate the association of FAI and PWV observed in our study.

Androgens, furthermore, are directly linked to CVD end points [18,35-37]. High levels of circulating testosterone in postmenopausal women have been associated with accelerated coronary atherosclerosis [36], as well as with increased prevalence [37] and incidence [18,35] of coronary heart disease. On the contrary, other studies support the hypothesis that women with lower levels of circulating testosterone have more carotid atherosclerosis compared with women with higher but normal levels of testosterone [16,20,21,35,36,38]. Low circulating testosterone, furthermore, has been associated with a

Table 3 – Linear regression analysis including arterial IMT as dependent variable and hormones as independent variables

IMT	CCA			Carotid arteries combined		
	R^2	β-Coefficient	P value	R^2	β-Coefficient	P value
Model I [a]						
E2 (pg/mL)	0.052	−0.001	.987	0.063	−0.125	.162
Δ4-A (ng/dL)	0.052	−0.014	.878	0.052	−0.068	.452
TESTO (ng/mL)	0.225	0.396	**<.001**	0.099	0.190	**.033**
SHBG (nmol/L)	0.083	−0.174	.050	0.048	−0.028	.753
DHEAS (μg/dL)	0.059	0.085	.340	0.047	−0.013	.885
FEI	0.052	−0.005	.960	0.052	−0.073	.413
FAI	0.137	0.262	**.003**	0.073	0.101	.258
Model II [b]						
E2 (pg/mL)	0.073	−0.029	.752	0.056	−0.137	.140
Δ4-A (ng/dL)	0.073	−0.003	.972	0.042	−0.065	.474
TESTO (ng/mL)	0.240	0.377	<.001	0.103	0.187	**.041**
SHBG (nmol/L)	0.110	−0.197	**.029**	0.040	−0.044	.640
DHEAS (μg/dL)	0.082	0.095	.292	0.038	−0.005	.957
FEI	0.073	−0.016	.857	0.043	−0.007	.422
FAI	0.178	0.283	**.002**	0.083	0.119	.201
Model III [c]						
E2 (pg/mL)	0.198	0.002	.983	0.197	−0.092	.302
Δ4-A (ng/dL)	0.198	−0.011	.898	0.193	−0.065	.461
TESTO (ng/mL)	0.326	0.376	**<.001**	0.222	0.189	**.035**
SHBG (nmol/L)	0.215	−0.151	.120	0.194	0.078	.423
DHEAS (μg/dL)	0.208	0.105	.265	0.189	0.003	.972
FEI	0.202	−0.070	.456	0.204	−0.132	.159
FAI	0.241	0.236	**.014**	0.189	0.009	.929

Bold indicates statistical significance: $P < .05$.
[a] Model I includes age + hormone.
[b] Model II includes model I + BMI and current smoking.
[c] Model III includes model II + HOMA-IR + HDL cholesterol + LDL cholesterol + triglycerides + SBP.

higher incidence and mortality of CVD in men [39]. In an attempt to explain the conflicting data on the association of endogenous androgens and CVD, Laughlin et al [35] proposed the hypothesis that, for optimal cardiovascular health, androgen levels should be within normal limits. Extremes of circulating androgens, whether high or low, may have a negative effect on the cardiovascular system. In the 20-year follow-up of the Rancho Bernardo cohort, women in the lowest and the highest quintile of bioavailable testosterone had odds ratio of 1.79 and 1.96, respectively, for developing symptomatic coronary heart disease compared with women in the middle quintile [35].

The hypothesis that androgens may exert a proatherogenic effect is further supported by studies on women with the polycystic ovary syndrome (PCOS), a well-described androgen excess condition [40]. Polycystic ovary syndrome has been directly connected to a proatherogenic state, linked to hyperandrogenism-related transcription of inflammatory mediators involved in atherogenesis, independent of obesity [41,42]. An increasing body of evidence suggests that these women have an increased risk of coronary heart and cerebrovascular disease in their postmenopausal years [43-45].

Testosterone's proatherogenic effect is supported by experimental and in vitro studies. Androgen exposure is associated with increased adhesion of mononuclear cells to the vascular endothelium and oxidation of LDL by mononuclear cell–derived macrophages [46,47]. Testosterone impairs the endothelium-dependent vasorelaxation in hypercholesterolemic rabbits [48]. Furthermore, testosterone has been associated with a decrease of the elastin to collagen ratio in aortic smooth muscle cell culture [49]. These mechanisms may mediate the positive association of serum testosterone with IMT and arterial stiffness observed in our study.

Lower levels of DHEAS were associated with increased arterial stiffness in our study. Accumulating evidence suggests that DHEAS may have a vasculoprotective role. Produced mainly by the adrenal glands and up to 20% by the ovaries, even after menopause [50], DHEAS has been widely associated with an antiproliferative effect by inhibition of important apoptosis pathways, such as the 3-phosphoinositide–dependent kinase pathway/nuclear factor of activated T-cells/hypoxia-inducible-factor axis [51]. Dehydroepiandrosterone has been found to be a potent vasodilator because it has been reported to increase the endothelial synthesis of NO by enhancing the expression and stabilization of endothelial NO synthase [52,53]. Finally, in another study, dehydroepiandrosterone decreased proliferation and increased vascular smooth muscle cell apoptosis both in vivo and in vitro [51,54]. On clinical grounds, a significant association between DHEAS levels and endothelial function was found in a postmenopausal population with known coronary risk factors [55]. In line with our results, a recent study in an elderly Japanese population with cardiovascular risk factors revealed a protective effect of DHEAS on IMT only in men, but not in women, whereas DHEAS was associated with increased carotid blood flow only in women [56]. Finally, recent data indicate that lower DHEAS levels may be related to higher cardiovascular

Table 4 – Linear regression analysis including indices of arterial function as dependent variables and hormones as independent variables

	FMD			PWV			AI		
	R^2	β-Coefficient	P value	R^2	β-Coefficient	P value	R^2	β-Coefficient	P value
Model I [a]									
E2 (pg/mL)	0.019	−0.095	.300	0.078	0.074	.409	0.020	0.076	.412
Δ4-A (ng/dL)	0.020	0.102	.267	0.092	0.140	.117	0.014	−0.020	.830
TESTO (ng/mL)	0.022	−0.111	.227	0.076	0.057	.526	0.014	−0.011	.905
SHBG (nmol/L)	0.014	0.064	.489	0.105	−0.180	**.042**	0.018	0.065	.483
DHEAS (μg/dL)	0.019	0.095	.302	0.100	0.165	.063	0.065	−0.227	**.013**
FEI	0.023	−0.115	.212	0.116	0.210	**.017**	0.025	0.104	.257
FAI	0.015	−0.074	.422	0.129	0.239	**.007**	0.020	0.080	.382
Model II [b]									
E2 (pg/mL)	0.019	−0.091	.344	0.117	0.039	.671	0.033	0.089	.350
Δ4-A (ng/dL)	0.022	0.100	.282	0.137	0.147	.095	0.026	−0.019	.834
TESTO (ng/mL)	0.023	−0.109	.250	0.118	0.046	.612	0.026	−0.016	.863
SHBG (nmol/L)	0.015	0.059	.536	0.134	−0.139	.124	0.027	0.044	.648
DHEAS (μg/dL)	0.022	0.103	.272	0.134	0.135	.129	0.071	−0.217	**.019**
FEI	0.023	−0.111	.243	0.146	0.176	**.048**	0.040	0.125	.187
FAI	0.016	−0.069	.476	0.152	0.198	**.028**	0.037	0.111	.246
Model III [c]									
E2 (pg/mL)	0.064	0.012	.914	0.177	0.020	.856	0.050	0.088	.477
Δ4-A (ng/dL)	0.140	0.187	.127	0.192	0.123	.301	0.085	−0.002	.991
TESTO (ng/mL)	0.144	−0.097	.315	0.218	0.070	.518	0.043	0.076	.548
SHBG (nmol/L)	0.097	−0.088	.487	0.246	−0.191	.103	0.052	0.054	.692
DHEAS (μg/dL)	0.095	0.013	.907	0.216	0.159	.141	0.103	−0.267	**.029**
FEI	0.108	0.090	.461	0.181	0.155	.092	0.045	0.051	.701
FAI	0.099	−0.025	.839	0.270	0.254	**.027**	0.044	0.117	.395

Bold indicates statistical significance: $P < .05$.

[a] Model I includes age + hormone.

[b] Model II includes model I + BMI and current smoking.

[c] Model III includes model II + HOMA-IR + HDL cholesterol + LDL cholesterol + triglycerides.

mortality in postmenopausal women with CVD risk factors undergoing coronary angiography for suspected ischemia [57].

Our study bears certain limitations. The sample size and the high dispersion of serum hormone values may not have provided enough statistical power to identify the presence of other significant associations. In addition, we did not measure estrone, which might be a more representative index of circulating estrogens. Furthermore, this study evaluates surrogate markers of CVD and not hard end points in a cross-sectional design, which does not permit the detection of causality. Another limitation of the study could be the multiple associations investigated, some of which might have occurred by chance. The associations, however, between IMT and testosterone remained significant even after Bonferroni correction for multiple comparisons. Finally, although we excluded women with a history of hirsutism, menstrual irregularities, or long-standing infertility, the inclusion of some cases of women with PCOS may have influenced our results [58]. On the other hand, a significant strength of this study is the homogeneity of the study population, derived by carefully applying exclusion criteria, without confounding factors known to affect the measured vascular markers such as long-standing menopause, hormone use, antihypertensive and lipid-lowering therapy, clinically overt CVD, or diabetes mellitus.

In conclusion, circulating testosterone is associated with both subclinical atherosclerosis and arterial stiffness, independently of age, BMI, lipids, and insulin resistance, indicating a possible direct association. This finding is important, as there are many states of androgen excess, like the PCOS, which, contrary to what was once believed, persists through menopause [59]. Women with PCOS receive clinical attention in reproductive years but usually are neglected once they become postmenopausal. If the causality of endogenous testosterone and atherosclerosis is proven, the documentation of elevated androgens as a risk factor for CVD will have important implications with regard to primary prevention policies.

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Conflict of Interest

No conflicts of interest.

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