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FULL RESEARCH PAPER
Biomarkers

Sex steroids and markers of micro- and macrovascular damage among women and men from the general population

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Aims The contribution of sex hormones to micro- and macrovascular damage might differ among women and men. In particular, little is known about the association between sex hormones and small vessel disease. Therefore, we examined the association of total oestradiol, total testosterone, free-androgen index (FAI), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione levels with micro- and macrovascular diseases.

Methods and results This cross-sectional study included 2950 women and 2495 men from the population-based Rotterdam Study. As proxy of microvascular damage, we measured diameters of retinal arterioles and venules. Markers of macrovascular damage included carotid intima-media thickness and carotid plaque, coronary artery calcification (CAC), and peripheral artery disease. Linear and logistic regression models were used and adjusted for age, cardiovascular risk factors, and years since menopause. *Associations with microvasculature:* In women, total testosterone [mean difference per 1-unit increase in natural-log transformed total testosterone (95% confidence interval, CI): 2.59 (0.08–5.09)] and androstenedione [4.88 (1.82–7.95)] and in men DHEAS [2.80 (0.23–5.37)] and androstenedione [5.83 (2.19–9.46)] were associated with larger venular caliber. *Associations with markers of large vessel disease:* In women, higher total testosterone [-0.29 (-0.56 to -0.03)], FAI [-0.33 (-0.56 to -0.10)], and androstenedione levels [-0.33 (-0.64 to -0.02)] were associated with lower CAC burden and FAI [odds ratio (95% CI): 0.82 (0.71–0.94)] was associated with lower prevalence of plaque.

Conclusion A more androgenic profile was associated with more microvascular damage in both women and men. Among women, however, higher androgen levels were also associated with less macrovascular damage. Our findings suggest that androgens might have distinct effects on the vasculature, depending on the vascular bed and stages of the atherosclerosis process.

Keywords Epidemiology • Cardiovascular disease • Atherosclerosis • Microvessels • Oestrogens • Androgens • Sex hormone-binding globulin • Biomarkers • Women

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Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide among women and men.[1,2] Both small vessel disease and large vessel disease are implicated in CVD pathophysiology and are predictors of future CVD risk.[3,4] While large vessel disease is considered to be the prominent underlying mechanism in the pathophysiology of CVD among men,[5] recent evidence suggests microvascular damage to play an important role in the pathophysiology of CVD in women.[6]

Parallel to menopausal transition, the risk of CVD increases in women.[7] Therefore, multiple studies have investigated the association between sex hormones and CVD risk.[8] However, the results regarding the impact of oestradiol and androgen levels on CVD risk remain controversial.[9] Since both micro- and macrovascular damage contribute to CVD pathophysiology,[3,4] these controversial results might reflect differing impact of sex hormones on small and large vessels. Moreover, contribution of sex hormones to micro- and macrovascular damage might also differ among women and men. However, little is known about the association between sex hormones and small vessel disease.

Therefore, we examined the association of total oestradiol, total testosterone, free-androgen index (FAI), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione levels with surrogate markers of micro- and macrovascular disease among women and men from the population-based Rotterdam Study. As the retina is an easy accessible window to assess the microvasculature, retinal vessel calibers were used as markers of microvascular disease. We included carotid intima-media thickness (IMT) and plaque, coronary artery calcification (CAC), and peripheral artery disease (PAD) as markers of large vessel disease.

Methods

Study population

This study was embedded within the Rotterdam Study, an ongoing prospective, population-based cohort study among individuals of 55 years and older living in the Ommoord district of Rotterdam, The Netherlands. The rationale and study design have been described in detail elsewhere.[10] The baseline examination of the Rotterdam Study included 7983 individuals between 1989 and 1993 (Rotterdam Study I) and has been extended twice (3011 individuals, Rotterdam Study II in 2000 and 3932 individuals, Rotterdam Study III in 2006) to include participants who were 45 years or older or who had moved to the study research area. Participants have been followed-up ever since and the examinations have been repeated every 3–4 years. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the 'Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)'.

This study included participants from the third visit of the original cohort (Rotterdam Study I-3, 1997–1999) and the from first visit of the extended cohort (Rotterdam Study III-1, 2006–2008) from whom measurements of markers of micro- and macrovascular damage as well as sex hormones were available ($N = 6867$). The response was 75.8% (4785 of 6315) for Rotterdam study cohort I-3 and 64.9% (3932 of 6059) for Rotterdam study III-1. Participants without informed consent ($N = 49$) and perimenopausal women or women for whom data on menopause age were missing ($N = 561$) were excluded. Among these participants, 2274 had microvascular measurements, 5215 had IMT, 5058 had carotid plaque measurements, 1524 had CAC measurements, and 2869 had PAD measurements (Supplementary material online, *Figure S1*).

Measurements of sex steroids

All blood samples were drawn in the morning (≤11 am) and were fasting. Total oestradiol levels were measured with COBAS 8000 Modular Analyzer (Roche Diagnostics GmbH), serum levels of total testosterone with liquid chromatography–tandem mass spectrometry (LC-MS/MS). Sex hormone-binding globulin (SHBG) was measured as with the Immulite platform (Diagnostics Products Corporation Breda). The corresponding interassay coefficients of variations for total oestradiol, sex hormone-binding globulin, and total testosterone were <7%, <5%, and <5%. The minimum detection limit for oestradiol was 18.35 pmol/L. Undetectable oestradiol was scored as 18.35. The FAI was calculated in women as (total testosterone/sex hormone-binding globulin)*100.[11] DHEA, DHEAS, and androstenedione were measured on a Waters XEVO-TQ-S system (Waters, Milford, MA, USA) using the CHS^{TM} MSMS Steroids Kit (Perkin Elmer, Turku, Finland). The inter-assay coefficients of variation of DHEA, DHEAS and androstenedione were <6.5%.

Measures of microvascular damage

Retinal vascular calibers were measured with fundus photographs (20° field, Topcon Optical Company, Tokyo, Japan) centred on the optic disc after pharmacological mydriasis. For each participant, the image of the eye with the best quality was analysed with a semi-automated system (IVAN, University of Wisconsin-Madison, Madison, WI, USA).

For each participant, using the revised Knudtson–Parr–Hubbard formula summary values were calculated for absolute arteriolar and venular calibers in micrometers, summarized as central retinal arteriolar and venular equivalents, representing the average of the six largest arteriolar and venular calibers of that eye, respectively.[12,13] Because eyes may have different magnification due to refractive changes, we adjusted vessel measurements for possible magnification variations with Littmann formula to approximate absolute measures.[14] We verified in a random subsample of 100 participants that individual measurements in the left and right eye were similar. Measurements were performed by one rater, masked for participant characteristics. Pearson correlation coefficients for interrater and intrarater agreement ($n = 100$) were 0.85 and 0.86 for arteriolar calibers, and 0.87 and 0.87 for venular calibers, respectively.

Measures of macrovascular damage

Carotid intima-media thickness and carotid plaque

A 7.5 MHz linear array transducer with a duplex scanner (ATL UltraMark IV) was used for ultrasonography of both carotid arteries. On a longitudinal two-dimensional ultrasound image of the carotid artery, the near and far walls of the carotid artery were displayed as two bright white lines separated by a hypo-echogenic space. The measurements were performed off-line on images frozen on the R wave of the electrocardiogram, to gate to the end-diastole. The distance from the leading edge of the first bright line of the far wall (lumen–intima interface) to the leading edge of the second bright line (media–adventitia interface) indicated the IMT. Measurements of the common carotid artery IMT involved a length of 10 mm distal of the bulb. IMT was determined as the average of mean,

near- and far-wall IMT, providing the average of left and right common carotid IMT.[15]

The presence of plaques in the carotid artery was assessed by examining the ultrasonographic images of the common, internal and bifurcation sites of the left and right carotid artery for the presence of atherosclerotic lesions. Plaques were defined as a focal widening relative to adjacent segments, with protrusion into the lumen composed of either only calcified deposits or a combination of calcified and non-calcified material at any of the six sites.

Coronary artery calcification

CAC was assessed in the epicardial coronary arteries detected on EBT scans. Imaging was performed with a C-150 Imatron scanner (GE-Imatron). Before participants were scanned, they exercised adequate breath-holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100-ms scan time and 3-mm slice thickness. Images were acquired at 80 of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Quantification of CAC was performed with AccuImage software (AccuImage Diagnostics Corporation), displaying all pixels with a density above 130 Hounsfield Units A calcification was defined as a minimum of 2 adjacent pixels with a density over 130 Hounsfield units.[16] A CAC score was calculated according to Agatston's method.

Peripheral artery disease

The method of measuring the ankle-brachial index has been described previously.[17] In short, (systolic) blood pressure in the arm was determined by calculating the mean of two successive measurements at the right brachial artery while the participant was in a sitting position (using a random-zero sphygmomanometer). In addition, the systolic blood pressure of the posterior tibial artery was measured while the participant was in a supine position (using a random-zero sphygmomanometer and an 8-MHz continuous-wave Doppler probe (Huntleigh 500 D, Huntleigh Technology)). The ankle-brachial index was defined as the ratio of systolic blood pressure at the ankle to systolic blood pressure at the arm and was calculated for each leg. The presence of PAD was defined as an ankle-brachial index of 0.90 or less.

Cardiovascular risk factors

Data on cardiovascular risk factors were available and included in the analyses. The risk factors included systolic and diastolic blood pressure, blood pressure-lowering medication, total and high-density lipoprotein (HDL) cholesterol, lipid-lowering medication, waist–hip ratio, C-reactive protein (CRP), smoking, prevalent diabetes, carotid plaque, prevalent coronary heart disease (CHD), and years since menopause (in women only). Methods for assessment of these risk factors are provided in the Supplementary material online.

Statistical analysis

The characteristics of the study population stratified by sex were presented as mean (standard deviation) or median (25th–75th quartile) for continuous variables and number (percentage) for categorical variables. Sex hormones, IMT and CAC were natural log transformed to approximate normal distribution. All analyses were performed separately for women and men.

Linear regression models were used to investigate the associations for linear outcomes (i.e. arteriolar and venular caliber, IMT, and CAC) and logistic regression models for categorical outcomes (i.e. presence of carotid plaque and PAD). Models were adjusted for age, systolic and diastolic blood pressure and blood pressure-lowering medication, total and HDL cholesterol, lipid-lowering medication, waist–hip ratio, CRP, smoking, prevalent diabetes, carotid plaque (for analyses of markers of microvascular damage), prevalent CHD (for analyses of markers of macrovascular damage), and years since menopause (in women only). We used data from two cohorts of the Rotterdam Study (I-3 and III-1) and meta-analysed the cohort-specific regression coefficients using the random effects model.

As a sensitivity analysis, we repeated the analyses among participants without prevalent CHD, chronic kidney disease (CKD) or diabetes. Next, we also repeated the analyses among women who had experienced natural menopause and participants without current use of hormones.

A two-sided *P*-value of <0.05 was regarded as statistically significant. Although the analyses for the markers of micro- and macrovascular damage can be considered as exploratory analysis, as a sensitivity analysis, we also considered a more conservative *P*-value by applying the Bonferroni correction for four exposure (total oestradiol, total testosterone and FAI, DHEA and DHEAS, AD) and four outcome (retinal vessel caliber, IMT and plaque, CAC, and PAD).

All analyses were performed using IBM SPSS Statistics version 24.0 (SPSS Inc., Chicago, IL, USA), R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria), and STATA version 15.1 (Stata Corp, College Station, TX, USA).

Results

Characteristics of the study population

Table 1 shows the characteristics of the 2950 women and 2495 men included in the analyses of large vessel disease. The mean age was 66.7 years for women and 64.4 years for men. Supplementary material online, *Table S1* presents the characteristics of 1159 women and 1115 men included for the analyses of small vessel disease.

The associations of sex hormones with micro- and macrovascular damage in women and men are shown in *Tables 2* and *3*, respectively.

Associations of sex hormones with markers of microvascular damage

In women, higher levels of total testosterone [adjusted mean difference (95% confidence interval, CI) per 1 unit increase in naturel-log transformed total testosterone: 2.59 (0.08–5.09)] and androstenedione [adjusted mean difference (95% CI): 4.88 (1.81–7.95)] and in men higher levels of DHEAS [adjusted mean difference (95% CI): 2.80 (0.23–5.37)] and androstenedione [adjusted mean difference (95% CI): 5.83 (2.19–9.47)] were associated with larger retinal venular caliber. Higher total oestradiol levels were associated with increase in retinal venular caliber in both women and men, albeit non-significantly [adjusted mean difference (95% CI) in retinal venular caliber: 1.34 (-0.19 to 2.86); *P*-value: 0.09] in women and [2.38 (-0.60 to 5.37); *P*-value: 0.12 in men].

No statistically significant associations were found for retinal arteriolar caliber in both sexes. In women androstenedione [adjusted mean difference (95% CI) in arteriolar caliber diameter: -1.57 (-3.56 to 0.41); *P*-value: 0.12] and among men DHEAS [adjusted mean difference (95% CI) in arteriolar caliber diameter: -1.35 (-2.98 to 0.28); *P*-value: 0.10] were also associated with decrease in arteriolar caliber, albeit non-significantly.

Table I Characteristics of the study population for the analysis of large vessel disease

	Women (*N* = 2950)	Men (*N* = 2495)
Age (years)	66.73 (9.38)	64.36 (9.67)
Body mass index (kg/m^2)	27.46 (4.60)	27.11 (3.64)
Waist–hip ratio	0.86 (0.09)	0.95 (0.08)
Current smoking, *N* (%)	546 (18.5)	649 (26.0)
Diastolic blood pressure (mmHg)	77.64 (11.57)	80.09 (11.61)
Systolic blood pressure (mmHg)	138.17 (21.27)	139.84 (19.96)
Antihypertensive therapy, *N* (%)	1046 (35.5)	850 (34.1)
Total cholesterol (mmol/L)	5.93 (1.00)	5.45 (0.98)
High-density lipoprotein cholesterol (mmol/L)	1.54 (0.42)	1.24 (0.33)
Lipid lowering medication, *N* (%)	500 (16.9)	502 (20.1)
C-reactive protein (mg/L)	1.91 (0.90–3.81)	1.70 (0.70–3.60)
Prevalent diabetes, *N* (%)	320 (10.8)	351 (14.1)
Prevalent coronary heart disease, *N* (%)	100 (3.4)	314 (12.6)
Prevalent chronic kidney disease, *N* (%)	393 (13.5)	264 (10.7)
Sex steroids		
Total oestradiol (pmol/L)	22.27 (18.35–41.88)	89.84 (71.62–112.60)
Total testosterone (nmol/L)	0.83 (0.59–1.17)	17.06 (13.45–21.39)
Free-androgen index	1.40 (0.94–2.09)	NA
Dehydroepiandrosterone (nmol/L)	9.83 (6.11–15.28)	9.59 (6.24–15.07)
Dehydroepiandrosterone sulfate (nmol/L)	1650.90 (1010.08–2589.18)	2871.32 (1751.08–4371.02)
Androstenedione (nmol/L)	2.37 (1.71–3.22)	3.00 (2.26–3.96)
Women-specific variables		
Age at menopause (years)	48.55 (5.64)	NA
Time since menopause (years)	18.17 (10.45)	NA
Natural menopause, *N* (%)	2240 (76.2)	NA
Markers of macrovascular damage		
Carotid intima-media thickness (mm)	0.99 (0.18)	1.03 (0.21)
Carotid plaque, *N* (%)	1813 (66.5)	1825 (78.2)
Coronary artery calcification, Agatston score	253.38 (569.85)	748.77 (1208.15)
Peripheral artery disease, *N* (%)	266 (16.2)	221 (18.0)
Markers of microvascular damage		
Arteriolar caliber (μm)	157.43 (15.18)	158.38 (15.42)
Venular caliber (μm)	236.98 (22.57)	240.95 (22.57)
Arterio-venular ratio	0.67 (0.06)	0.66 (0.06)

Values are reported as number (percentage) for categorical variables and mean (SD) or median (25th–75th quartile) for continuous variables.
N, number; NA, not applicable.

Associations of sex hormones with markers of macrovascular damage

In women, higher levels of total testosterone [adjusted mean difference (95% CI) per 1 unit increase in naturel-log transformed total testosterone: -0.29 (-0.56 to -0.03)], FAI [-0.33 (-0.56 to -0.10)], and androstenedione levels [-0.33 (-0.64 to -0.02)] were associated with lower CAC burden and higher FAI [adjusted odds ratio (95% CI): 0.82 (0.71–0.94)] was associated with lower prevalence of carotid plaque.

In men, albeit non-significant, higher levels of total testosterone were associated with lower CAC burden [adjusted mean difference (95% CI): -0.20 (-0.45 to 0.06), *P*-value:0.13], higher androstenedione levels was associated with a higher prevalence of carotid plaque [adjusted odds ratio (95% CI): 1.30 (0.99–1.69), *P*-value:0.05], and higher levels of DHEA was associated with higher prevalence of PAD [adjusted odds ratio (95% CI): 1.24 (0.95–1.63), *P*-value: 0.12].

Sensitivity analysis

Overall, after repeating the analyses in persons free of CHD, CKD, and diabetes (Supplementary material online, *Table S2a* and *b*), among women with natural menopause (Supplementary material online, *Table S3*) and after excluding participants currently using hormones (Supplementary material online, *Table 4a* and *b*), the results were overall in line with the results from the total population. However, some estimates attenuated.

After applying a strict Bonferroni correction ($P = 0.003$), the association between androstenedione and venular caliber among both sexes remained significant.

Table 2 Association between sex steroids and markers of micro- and macrovascular damage among women

	Total oestradiol	Total testosterone	Free-androgen index	DHEA	DHEAS	Androstenedione
	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Small vessels						
Arteriolar caliber	-0.38 (-1.37; 0.60)	-0.92 (-2.53; 0.69)	0.06 (-1.25; 1.38)	-0.46 (-1.53; 0.61)	-1.02 (-2.43; 0.40)	-1.57 (-3.56; 0.41)
Venular caliber	1.34 (-0.19; 2.86)	**2.59 (0.08; 5.09)**	-0.76 (-2.80; 1.28)	0.63 (-1.04; 2.29)	0.87 (-1.33; 3.07)	**4.88 (1.82; 7.95)**
Large vessels						
IMT[a]	0.01 (-0.01; 0.02)	0.01 (-0.003; 0.02)	0.002 (-0.01; 0.01)	0.001 (-0.01; 0.01)	0.01 (-0.01; 0.02)	0.002 (-0.01; 0.01)
CAC	0.08 (-0.24; 0.39)	**-0.29 (-0.56; -0.03)**	**-0.33 (-0.56; -0.10)**	-0.18 (-0.42; 0.07)	-0.07 (-0.28; 0.14)	**-0.33 (-0.64; -0.02)**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Carotid plaque[a]	0.95 (0.83; 1.10)	0.95 (0.81; 1.13)	**0.82 (0.71; 0.94)**	1.03 (0.90; 1.18)	1.01 (0.89; 1.14)	1.05 (0.88; 1.24)
Peripheral artery disease	0.90 (0.67; 1.21)	1.12 (0.86; 1.44)	0.88 (0.71; 1.10)	0.89 (0.71; 1.11)	1.00 (0.83; 1.21)	1.06 (0.81; 1.40)

Values are adjusted mean difference (95% confidence intervals) for retinal vessel caliber, IMT and CAC and odds ratio's (95% confidence intervals) for presence of carotid plaque and peripheral artery disease. Sex hormones, IMT, and CAC are natural log transformed.
Significant results ($P < 0.05$) are in bold.
Models were adjusted for age, systolic and diastolic blood pressure and blood pressure lowering medication, total and HDL cholesterol, lipid lowering medication, waist–hip ratio, C-reactive protein, smoking, prevalent diabetes, carotid plaque (for analyses of markers of microvascular damage), prevalent coronary heart disease (for analyses of markers of macrovascular damage), and years since menopause (in women only).
Models included for small vessels $N = 1159$, for IMT $N = 2825$, CAC $N = 805$, carotid plaque $N = 2725$, and peripheral artery disease $N = 1644$.
CAC, coronary artery calcification; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate; IMT, carotid intima-media thickness; *N*, number.
[a]Pooled estimate of the IMT and carotid plaque analyses for Rotterdam Study I and Rotterdam Study III.

Table 3 Association between sex steroids and markers of micro- and macrovascular damage among men

Women	Total oestradiol	Total testosterone	Free-androgen index	DHEA	DHEAS	Androstenedione
	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Small vessels						
Arteriolar caliber	-0.64 (-2.53; 1.26)	0.82 (-1.00; 2.64)	NA	-0.02 (-1.31; 1.27)	-1.35 (-2.98; 0.28)	-0.16 (-2.48; 2.17)
Venular caliber	2.38 (-0.60; 5.37)	1.14 (-1.75; 4.02)	NA	0.49 (-1.54; 2.53)	**2.80 (0.23; 5.37)**	**5.83 (2.19; 9.46)**
Large vessels						
IMT[a]	0.01 (-0.01; 0.03)	-0.01 (-0.02; 0.004)	NA	0.004 (-0.01; 0.02)	0.003 (-0.01; 0.01)	0.004 (-0.01; 0.02)
CAC	-0.25 (-0.62; 0.11)	-0.20 (-0.45; 0.06)	NA	-0.09 (-0.33; 0.15)	-0.16 (-0.37; 0.06)	-0.09 (-0.43; 0.24)
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Carotid plaque[a]	1.18 (0.89; 1.55)	1.02 (0.85; 1.31)	NA	1.03 (0.85; 1.24)	1.06 (0.88; 1.27)	1.30 (0.99; 1.69)
Peripheral artery disease	1.07 (0.68; 1.66)	0.98 (0.73; 1.32)	NA	1.24 (0.95; 1.63)	1.09 (0.86; 1.39)	1.17 (0.80; 1.69)

Values are adjusted mean difference (95% confidence intervals) for retinal vessel caliber, IMT and CAC and odds ratio's (95% confidence intervals) for presence of carotid plaque and peripheral artery disease. Sex hormones, IMT, and CAC are natural log transformed.
Significant results ($P < 0.05$) are in bold.
Models were adjusted for age, systolic and diastolic blood pressure and blood pressure lowering medication, total and HDL cholesterol, lipid lowering medication, waist–hip ratio, C-reactive protein, smoking, prevalent diabetes, carotid plaque (for analyses of markers of microvascular damage), and prevalent coronary heart disease (for analyses of markers of macrovascular damage).
Models included for small vessels $N = 1115$, for IMT $N = 2390$, CAC $N = 719$, carotid plaque $N = 2333$, and peripheral artery disease $N = 1225$.
CAC, coronary artery calcification; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate; IMT, carotid intima-media thickness; N, number.
[a]Pooled estimate of the IMT and carotid plaque analyses for Rotterdam Study I and Rotterdam Study III.

Discussion

We investigated the association between sex hormones and several markers of micro- and macrovascular damage. We reported a significant association between higher androgen levels and more microvascular damage, as reflected in larger retinal venular caliber, in both women and men. Among women, higher androgen levels were associated with lower burden of macrovascular damage.

Markers of microvascular damage

Retinal vessel calibers have been widely used as a marker of microvascular disease, as the retina is an easy accessible window for non-invasive assessment of microvasculature *in vivo*. Therefore, the retinal microvasculature provides a unique platform to assess the state of the microcirculatory system in the body. We observed an association between larger venular caliber with higher total testosterone and androstenedione levels in women and with DHEAS and androstenedione among men. Also, in both sexes, higher total oestradiol levels showed borderline significant associations with larger venular caliber. Larger venular caliber is a marker of more severe microvascular damage. Androgens have been found to be a potent vasodilator, by decreasing the endothelial synthesis and activation of nitric oxide and enhancing the expression and stabilization of endothelial nitric oxide synthesis.[18,19] Prior research has also shown an association between androgens with other markers of microvascular disease. A previous study among women with polycystic ovary syndrome (PCOS) showed that higher levels of androgens were associated with microvascular endothelial dysfunction[20] and impaired microvascular dilation.[21] Another study showed that among hypogonadal men receiving androgen treatment impairs microvascular function assessed in the forearm.[22] Similar to our study, several studies have shown a vasodilatory effect of oestradiol on vessels.[23]

We did not find any associations between sex hormones with retinal arteriolar caliber. Tissue-specific effect of sex hormones might partly explain why we found overall strong associations for venular caliber and not for arteriolar caliber. However, retinal arteriolar and venular caliber may also differently reflect the effect of various risk factors and pathophysiological mechanisms and might carry different information. Previous epidemiological studies have shown that arteriolar and venular caliber are associated with different systemic diseases, morbidities and pathological conditions.[24]

One study found narrower arteriolar caliber to be associated with cardiovascular mortality, whereas venular widening was also associated with non-CVD.[25] While retinal arteriolar narrowing has shown strong associations with high blood pressure, larger retinal venular widening has been associated with for instance systemic inflammation.[24] Moreover, the MESA study found a significant association between venular widening, but not arteriolar caliber, with brachial endothelial dysfunction, suggesting that retinal venular dilatation could be a marker of (systemic) endothelial dysfunction.[26] Although the exact pathophysiological mechanisms underlying venular dilatation remain to be elucidated, previous studies suggested a role for inflammation and endothelial dysfunction as important underlying mechanisms of venular dilatation.[27,28]

Markers of macrovascular damage

Higher levels of androgens were associated with less macrovascular damage among women in our study. There were varying associations of androgens with markers of large vessel damage among women. Multiple previous studies have shown similar results; as low levels of total testosterone have been associated with more severe carotid atherosclerosis in women[29,30] and men.[31,32] In contrast to our study, a higher androgenic profile was associated with increased risk of CAC in MESA cohort.[33] Previous study has shown that coronary and carotid atherosclerosis might reflect two distinct processes.[34] Whether this could explain varying associations of androgens with coronary and carotid measures in men, albeit statistically non-significant, needs further investigation.

Moreover, the results regarding the association of sex steroids and SHBG with incident clinical CVD remains conflicting. In a previous study on women with postmenopausal remnant features of PCOS, we showed that associations of sex steroids with atherosclerosis markers did not translate into a significant clinical risk for incident CVD among these women.[35]

Conflicting results could, at least partly, be explained by the differences in: (i) characteristics of the study population (e.g. mean age, race and underlying comorbidity and cardiovascular risk) and (ii) use of different methods and assays to measure sex steroids and SHBG and (iii) varying adjustments for cardiovascular risk factors. In fact, part of the controversy in the results could be expected based on the timing hypothesis[36] and the concept that associations with sex hormones may differ depending on the underlying cardiovascular risk in the studied population.[37,38] Prior studies have also shown associations between sex hormones and general health status[39] and chronic diseases.[40] To address this issue, we included a sample of the general population in our study and further performed sensitivity analyses in different subpopulations free of comorbidities at baseline.

Varying associations of sex hormones with markers of micro- and macrovascular damage

Our analysis in a large population-based cohort study showed associations in differing directions for androgens with retinal venular caliber and CAC and carotid plaque in women. While CAC and the presence of carotid plaques are considered indices of more advanced atherosclerosis, retinal vessel caliber is suggested as an early marker of atherosclerosis. As such, a potential explanation for the observed differential results might be dependency of the impact of sex hormones on the stage of atherosclerosis as hormone receptor expression varies in different stages of atherosclerosis and progression of atherosclerosis.[38] Other previous studies have similarly observed differential effects depending on the vascular bed under study.[41] In a previous animal study among male swine, androgens showed differential effect on the coronary blood flow regulation of the coronary microvessels and the arteriolar caliber.[42]

While no association was found for oestradiol in association with markers of large vessel disease, total oestradiol showed associations with retinal venular caliber, albeit statistically non-significant. The lack of strong associations might be due to lack of enough power of this study for markers of small vessel disease, as relatively large effect sizes with borderline significance were observed for both women and men. Furthermore, small vessels have a relative small size. As such, observing significant differences are more difficult within small sample sizes. Moreover, oestradiol might also exert varying impact on vessels depending on the atherosclerosis stage. It has been suggested that higher oestrogen levels might have a role in preventing atherosclerosis but may aggravate progression among those with established disease.[38] A previous study has also shown differential effect of total oestradiol on vascular properties (i.e. carotid diameter and distensibility).[43] Furthermore, although sex hormone receptors are both found on endothelial cells and smooth muscle cells, sex hormones

may have a divergent effect depending on the cell type.[27] Since the microvessels and macrovessels differ in their smooth muscle cell and endothelial cell content, the actions of the sex steroids on microvessels and macrovessels may be due to the cellular composition of the vessels.[27] Another potential explanation may be that we used single hormonal measurement while long-term exposure, including cumulative exposure and total duration of hormone exposure, might be more important and this was not assessed in our study. Furthermore, total oestradiol levels are low in postmenopausal women, leading to a low interindividual variability of total oestradiol in these women. This could make it more difficult to find a significant association. In addition, previous studies have suggested that hormone imbalance (i.e. oestradiol–testosterone ratio), rather than absolute hormone values, may be important for increased cardiovascular risk, however, little is known on this matter.[44] Oestrogen generated from high levels of testosterone may influence atherosclerosis development. Nevertheless, the complex interplay and interaction between the hormones remains unknown.

Differences between women and men

Recent literature suggests microvascular disease to be more prevalent and a more dominant underlying mechanism for CVD among women compared to men. However, the association of sex hormones with markers of microvascular damage in our study was similar for both sexes. Both total oestradiol and androgen levels showed associations with microvascular damage in women and men. For markers of macrovascular damage, more associations with sex hormones were found in women compared to men. This might suggest that in both women and men sex hormones are important for both micro- and macrovascular damage and that the sex differences in prevalence and dominance of microvascular damage in women might be due to other factors. However, several studies have suggested that microvascular damage may be more important in younger women. Therefore, the observed sex differences on microvascular disease might narrow after the menopause.[45] This may explain why we did not find differences between women and men with regard to the impact of sex hormones on microvascular damage.

Pathological interactions between microvascular and macrovascular damage are common and similar mechanisms, including shared risk factors, drive the development and progression of both small and large vessel disease.[46] Sex differences in the strength of the associations of other, either common or as yet unknown, risk factors with markers of small and large vessel disease and their interactions with sex hormones are plausible.

Differences between study populations

When restricting the analysis to a population free of CHD, CKD, and diabetes mellitus (DM), the association between sex hormones with venular caliber, CAC, and PAD attenuated. A possible explanation for this finding could be the smaller sample size used for this analysis. However, overall the effect sizes were also slightly smaller in the healthy population free of CHD, CKD, and DM. This may also suggest that in individuals with 'healthy' vascular ageing, the impact of sex hormones on vasculature may be to a lesser extent. This is in line with prior studies, in both humans and animals, that suggested that the effect of sex hormones could be different among individuals with and without atherosclerosis and thus vary based on underlying cardiovascular risk.[36,38] However, we also cannot exclude that in a population free of atherosclerotic disease there may be no association between sex hormones with markers of vascular damage.

Strength and limitations

The major strengths of this study include the large community-dwelling study sample, availability of a broad range of sex hormones in both women and men, availability of both markers of small and large vessel disease, and detailed assessment of a broad range of cardiovascular risk factors. Moreover, all blood samples were by protocol drawn in the morning, which is important due to the circadian variation of sex hormone levels. Finally, androgens were measured using the golden standard method. Limitations of this study include the cross-sectional design that does not allow for conclusions regarding causality. Additionally, markers of micro- and macrovascular damage were measured in different sub-cohorts of the Rotterdam study. As such, measurements of both micro- and macrovascular damage were not available in all participants. However, for participants for whom both data of micro- and macrovascular damage was available, measurements were performed in the same visit. Moreover, we included middle-aged and elderly (postmenopausal) women and men from a community-dwelling population, with a mean age of 66 years. Our results are generalizable to other populations with similar characteristics. Prior studies have shown that sex hormone and SHBG levels differ in women and men from different age-groups[48–50] and may exert a different effect in different age-groups, as the effect of sex hormone may also differ by cardiovascular risk profiles at different age-groups.[37] As such, our results should not be automatically generalized to e.g. younger populations. Also, in this study, we included a representative sample from a general population, mainly from European ancestry. Although our results can be extrapolated to wide European populations, this cannot be done for other ethnicities.[51,52] Finally, the immunoassay to measure oestradiol had a minimum detection limit of 18.35 pmol/L.

Conclusions

This is the first study investigating the association between total oestradiol and androgens with markers of both micro- and macrovascular damage among women and men from general population. A more androgenic profile was associated with more microvascular damage in both sexes. Among women, however, higher androgen levels were also associated with less macrovascular damage. Our findings suggest that androgens might have distinct effects on the vasculature, depending on the vascular bed and stages of the atherosclerosis process. Further investigations are needed to clarify the underlying mechanisms of the vessel-specific and sex-specific effects of sex hormones on the vasculature.

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology* online.

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Authors' contributions

E.A., J.R.V.L., and M.K. contributed to the conception or design of the work. E.A., U.M., M.K.I., D.B., C.C.W.K., M.A.I., J.R.V.L., and M.K. contributed to the acquisition, analysis, or interpretation of data for the work. E.A. drafted the manuscript. F.A., U.M., M.K.I., D.B., J.S.E.L., C.C.W.K., M.A.I., J.R.V.L., and M.K. critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Conflict of interest The Author(s) declare(s) that there is no conflict of interest.

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