Endogenous sex hormones and subclinical atherosclerosis in middle-aged and older men ☆

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ARTICLE INFO

Article history:
Received 17 December 2012
Accepted 16 January 2013
Available online 7 March 2013

Keywords:
Sex hormones
Subclinical atherosclerosis
Pulse wave velocity
Carotid intima-media thickness
Plaque score

Circulating sex hormone levels have been linked to a wide range of cardiovascular risk factors in men, but studies on incident CVD have been inconclusive [1]. Recent data from meta-analyses show an increase in CVD risk with low testosterone in elderly men and no association with estradiol levels [2,3]. To clarify the role of sex hormones in male CVD risk, we examined the cross-sectional and longitudinal associations between endogenous sex hormones and subclinical atherosclerosis in a population-based cohort of middle-aged and older men.

For this analysis we included data from 400 independently living men aged 40 to 80 years, of whom 270 underwent a re-examination after a median follow-up of 8.8 years. Details of the study design and measurements have been reported previously [4]. At baseline, fasting morning blood samples and data on anthropometric parameters, blood pressure, medical history and lifestyle factors were collected. Serum levels of total testosterone (TT), dihydroepiandrosterone sulphate (DHEAS), total estradiol (E2) and sex hormone-binding globulin (SHBG) were measured and free levels of testosterone (FT) and estradiol (FE2) were calculated. We examined three atherosclerotic markers using noninvasive methods. Carotid intima-media thickness (cIMT) and pulse wave velocity (PWV) are markers of early atherosclerotic changes and were measured at baseline and follow-up using a 7.5-MHz linear array transducer (Acuson Aspen) and SphygmoCor device (PWV Medical, Sydney, Australia) respectively. At follow-up, we also evaluated carotid plaque burden as a marker of more advanced atherosclerosis using a 4-level rating scale: 0 = no plaque, 1 = minimal plaque, 2 = moderate plaque and 3 = severe plaque. The Institutional Review Board of the University Medical Center Utrecht approved the study and written informed consent was obtained from all participants. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

We used general linear models to assess the association of sex hormones with 1. cIMT and PWV at baseline; 2. Δ cIMT and PWV (defined as the absolute mean difference of cIMT and PWV divided by the follow-up time (years)) and 3. carotid plaque score at follow-up. Covariates included in the analyses were age, BMI, smoking status, alcohol consumption and antihypertensive and lipid-lowering medication. Analyses for PWV were additionally adjusted for mean arterial pressure and heart rate. Sex hormones were modelled per standard deviation (sd) decrease and tests for effect modification by age were performed using continuous interaction terms. We also analysed sex hormones in quartiles to examine possible non-linear associations.

Table 1 shows the baseline characteristics of the study population. The mean (sd) age at entry was 60 (11) years. Men who participated at follow-up were younger, consumed more alcohol, were more often never-smokers and had a lower prevalence of diabetes and cardiovascular disease at baseline. In addition, participants at follow-up had lower blood pressures, lower PWV and cIMT values and higher FT and DHEAS concentrations at baseline (Table 1). Table 2 shows the results of the cross-sectional and longitudinal analyses for PWV and cIMT. After multivariable adjustment we found no association between endogenous sex hormones and PWV and cIMT, neither for baseline measurements nor for change in PWV and cIMT during follow-up. Also, no association with plaque score at follow-up was observed (data not shown).

Table 1
Baseline characteristics of the total cohort and stratified by participation at follow-up.

	Total population at baseline	Participation at follow-up	No participation at follow-up
	(N = 400)	(N = 270)	(N = 130)
Age (years)	60.2 (11.3)	57.7 (10.6)	63.5 (11.0)
Body mass index (kg/m^2)	26.3 (3.5)	26.2 (3.4)	26.5 (3.6)
Waist circumference (cm)	98.9 (9.4)	98.3 (9.4)	100.1 (9.2)
Total testosterone (nmol/L)	18.5 (5.3)	18.6 (5.3)	18.5 (5.4)
SHBG (nmol/L)	40.6 (14.5)	39.7 (14.9)	42.4 (13.4)
Free testosterone (pmol/L)	354.2 (98.1)	361.0 (100.7)	340.1 (91.2)
Estradiol (pmol/L)	91.2 (22.8)	90.7 (22.4)	94.3 (23.8)
Free estradiol (pmol/L)	2.3 (0.5)	2.3 (0.6)	2.3 (0.6)
DHEAS (pmol/L)	6.7 (3.3)	7.1 (3.2)	5.7 (3.2)
Systolic blood pressure (mm Hg)	143.4 (22.1)	140.8 (20.3)	148.9 (24.6)
Diastolic blood pressure (mm Hg)	81.5 (10.3)	80.2 (10.1)	84.1 (10.2)
Total cholesterol (mmol/L)	5.8 (1.1)	5.9 (1.1)	5.7 (1.0)
Triglycerides (mmol/L)	1.6 (1.4)	1.6 (1.3)	1.6 (1.5)
Carotid IMT (mm)	0.82 (0.15)	0.80 (0.15)	0.87 (0.15)
Pulse wave velocity (m/s)	9.41 (2.49)	8.93 (2.17)	10.43 (2.82)
Mean arterial pressure (mm Hg)	129.1 (16.6)	126.9 (15.2)	134.0 (18.4)
Heart rate (mbp)	64.2 (10.2)	63.5 (10.5)	65.5 (9.6)
Smoking, % (N)			
Current	24.3 (97)	25.6 (69)	21.5 (28)
Former	54.3 (217)	50.0 (135)	63.1 (82)
Never	21.5 (86)	24.4 (66)	15.4 (20)
Alcohol consumption (g/day)	20.2 (21.5)	21.9 (23.2)	16.7 (17.2)
Cardiovascular disease, % (N)	17.0 (68)	14.1 (38)	23.1 (30)
Diabetes, % (N)	5.3 (21)	2.6 (7)	10.8 (14)
Hypertension therapy, % (N)	17.0 (68)	14.1 (38)	23.1 (30)
Hyperlipidemia therapy, % (N)	12.5 (50)	11.1 (30)	15.4 (20)

Values are expressed as mean (SD), unless stated otherwise. Abbreviations: SHBG = sex hormone-binding globulin; DHEAS = dihydroepiandrosterone sulphate.

☆ Funding: This study was financially supported by the International Health Foundation, Geneva, Switzerland and the Netherlands Organisation for Health Research and Development, grant 60-61900-98-146. None of the funding organisations had any role in the study design, collection, analysis and interpretation of the data, writing of the report or the decision to submit the paper for publication.

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Table 2
Cross-sectional and longitudinal associations between sex hormone levels and carotid intima media thickness and pulse wave velocity.

	Baseline cIMT (N = 399)		Baseline PWV (N = 376)		Δ cIMT (N = 265)		Δ PWV (N = 243)	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Total testosterone per sd decrease	0.003 (−0.01; 0.01)	−0.002 (−0.01; 0.01)	0.20 (−0.003; 0.40)	−0.01 (−0.20; 0.19)	−0.0004 (−0.002; 0.001)	−0.0003 (−0.002; 0.001)	0.004 (−0.02; 0.03)	0.02 (−0.02; 0.04)
SHBG per sd decrease	0.002 (−0.01; 0.01)	−0.001 (−0.01; 0.01)	0.22 (0.01; 0.43)	−0.02 (−0.22; 0.18)	−0.001 (−0.002; 0.001)	−0.001 (−0.002; 0.001)	−0.01 (−0.04; 0.01)	−0.002 (−0.03; 0.03)
Free testosterone per sd decrease	0.003 (−0.01; 0.02)	−0.0003 (−0.01; 0.01)	0.08 (−0.13; 0.29)	0.01 (0.18; 0.29)	0.0001 (−0.001; 0.001)	0.0003 (−0.001; 0.002)	0.01 (−0.01; 0.04)	0.01 (−0.01; 0.04)
Estradiol per sd decrease	−0.01 (−0.02; 0.002)	−0.01 (−0.02; 0.01)	−0.02 (−0.21; 0.18)	−0.01 (−0.19; 0.17)	−0.0001 (−0.001; 0.001)	0.0003 (−0.001; 0.002)	−0.01 (−0.03; 0.02)	−0.01 (−0.04; 0.01)
Free estradiol per sd decrease	−0.01 (−0.02; 0.001)	−0.01 (−0.02; 0.01)	−0.11 (−0.31; 0.09)	−0.002 (−0.18; 0.18)	0.0002 (−0.001; 0.001)	0.001 (−0.001; 0.002)	−0.002 (−0.03; 0.02)	−0.01 (−0.03; 0.02)
DHEAS per sd decrease	0.01 (−0.01; 0.02)	0.01 (−0.01; 0.02)	−0.13 (−0.36; 0.11)	−0.09 (−0.30; 0.12)	−0.0002 (−0.002; 0.001)	−0.0002 (−0.002; 0.001)	−0.02 (−0.04; 0.01)	−0.01 (−0.04; 0.02)

Model 1: age-adjusted.
Model 2: Model 1 plus BMI, smoking, alcohol consumption, antihypertensive medication and lipid-lowering medication (Model 2*: additionally adjusted for mean arterial pressure and heart rate).
Abbreviations: SHBG = sex hormone-binding globulin; DHEAS = dihydroepiandrosterone sulphate.

However, there was some indication of effect modification by age (P interaction < 0.05). At baseline, low SHBG and testosterone were associated with higher PWV in men aged >70 years only (betas per sd decrease of SHBG and TT were = 0.64 (95% CI: 0.09; 1.20) and 0.48 (95% CI −0.09; 1.06) respectively). Quartile analysis revealed no evidence of nonlinearity and all associations remained unchanged after excluding prevalent CVD cases and men using antihypertensive and lipid-lowering medication. Results were also not materially different after repeating the longitudinal analyses using inverse probability weighting, making selective loss to follow-up an unlikely explanation for our findings.

Results of the present study argue against a major role of endogenous estradiol in male CVD risk. The absence of an association with subclinical atherosclerosis is consistent with data on incident CVD [3]. By contrast, a link with androgens seems to be more plausible. Despite the lack of an overall association between testosterone and subclinical atherosclerosis in this study, which is in agreement with previous findings on cIMT in men of the same age range [5,6], low testosterone levels have been associated with atherosclerotic markers in selected populations of elderly men [7], hypogonadal men [8] and men with low-grade inflammation [9]. In line with this, we found evidence of effect modification by age in cross-sectional analyses for PWV, an interaction that has been reported previously for incident CVD [2]. Existing data on DHEAS are also supportive of an association in specific subpopulations [10,11], although we could not identify such an association in this study. The few studies on SHBG have yielded inconsistent results, with either a borderline significant association [5] or no association [9]. Our findings for SHBG, however, are in accordance with recent data showing an association between SHBG and cardiovascular events in elderly men [12].

Altogether, the findings of our and other studies suggest that, if present, the strength of an association is very small in apparently healthy men. Therefore, a more likely explanation for the observed associations in high-risk populations is reverse causation, in which underlying disease processes cause a decrease in testosterone and SHBG levels. Alternatively, the absence of an overall association may be indicative of a threshold effect, with low androgens only putting men at risk below a certain level. This hypothesis is supported by results from testosterone supplementation studies showing improvements in vascular function in hypogonadal men [13] and men with a history of cardiovascular disease [14], i.e. men having low testosterone levels.

In conclusion, our results do not support an overall association between endogenous sex hormones and subclinical atherosclerosis in middle aged and older men. Large-scale studies focussing on threshold effects are needed to gain more insight into the role of sex hormones in CVD aetiology.

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0167-5273/$ – see front matter
http://dx.doi.org/10.1016/j.ijcard.2013.01.242

Renal function, P-gp-affecting drugs and new anticoagulants for stroke prevention

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ARTICLE INFO

Article history:
Received 23 December 2012
Accepted 16 January 2013
Available online 8 February 2013

Keywords:
Atrial fibrillation
Renal failure
Anticoagulation

New oral anticoagulants (NOAs), like the thrombin-inhibitor dabigatran etexilate or the factor Xa-inhibitor rivaroxaban showed similar efficacy as the vitamin-K-antagonist (VKA) warfarin for stroke prevention in patients with atrial fibrillation (AF) [1,2]. One of the advantages of the NOAs, compared with VKAs, should be the lack of the necessity for laboratory monitoring and its lower rate of drug- and food interactions. The serum concentration of NOAs, however, is influenced by renal function. Furthermore, NOA-absorption is dependent on the intestinal P-glycoprotein (P-gp)-system, and P-gp activity is influenced by several drugs [3]. Since the prevalence of renal failure and the rate of patients taking P-gp-affecting drugs in AF are largely unknown, we performed a cross-sectional study to assess renal function and prescription-frequency of P-gp-affecting drugs, as searched from the literature in hospitalized AF-patients.

The charts of 100 consecutive patients, hospitalized between December 2009 and January 2010 were reviewed. The $CHADS_2$ score was calculated and the medication was screened for P-gp-affecting drugs and VKA-prescription. At that time no NOAs were prescribed in the department. The glomerular filtration rate was calculated by the "modification of diet in renal disease" formula.

In 100 patients (47 females, mean age 74 ± 12 years) heart failure was present in 53, hypertension in 70, diabetes in 33 and prior stroke in 14. The mean $CHADS_2$ score was 2.4 ± 1.4. The GFR was <30 ml/min/1.73 m^2 in 9 patients, 30–45 in 12 patients, 46–59 in 21 patients, 60–89 in 42 patients and ≥90 in 16 patients. VKAs received 54 patients: 2/8 with $CHADS_2$ 0, 8/20 with $CHADS_2$ 1, 15/27 with $CHADS_2$ 2, 15/24 with $CHADS_2$ 3, 10/14 with $CHADS_2$ 4, 2/5 with $CHADS_2$ 5 and 2/2 with $CHADS_2$ 6.

Forty-two patients took at least one, and 5 of the 42 patients took two P-gp-affecting drugs: Simvastatin (n = 22), amiodarone (n = 8), vitamin E (n = 8), carvedilol (n = 4), diltiazem (n = 2), dipyridamole (n = 1), propranolol (n = 1), verapamil (n = 1). Twenty-six of the 54 VKA-receiving patients took one (n = 23) or two (n = 3) P-gp-affecting drugs.

P-gp-affecting drugs were prescribed to 3 patients with GFR <30 ml/min/1.73 m^2, 4 patients with GFR 30–45, 9 patients with GFR 46–59, 18 patients with GFR 60–89 and 7 patients with GFR ≥ 90. Only 9 patients had a normal renal function and they had no prescription of P-gp-affecting drugs.

This retrospective cross sectional study in hospitalized patients indicates that renal impairment is a frequent finding in AF-patients. Our results from hospitalized patients show a higher prevalence of impaired renal function than a Swedish outpatients-registry where 4% of AF-patients had a GFR <30 ml/min/1.73 m^2 and 16% <45 ml/min/1.73 m^2 [4]. This discrepancy may be due to the higher comorbidity of hospitalized patients than of outpatients.

Patients with renal impairment are underrepresented in the above mentioned trials about NOAs in AF: Patients with severe renal impairment, defined as a creatinine clearance of <30 ml/min which is comparable to a GFR of <30 mL/min/1.73 m^2, were excluded [1,2]. The proportion of patients with creatinine clearance of <50 ml/min was in the present study 30%, and it was only 19% in the RE-LY trial investigating dabigatran, and 20% investigating in the ROCKET-AF trial investigating rivaroxaban [1,2]. Thus, the relatively low bleeding rate in RE-LY and ROCKET-AF may be partially explained by the low proportion of patients with severe renal failure. In the meantime, bleeding complications in several dabigatran-treated patients outside clinical trials with renal failure have been reported (Table 1) [5–9]. Of interest, most of the patients listed in the table were older than 80 years, whereas the mean age of the RE-LY patients was 71 years and that of the ROCKET-AF patients was 73 years [1,2].

P-gp is a product of the *MDR1* (multi drug resistance 1) gene and has considerable genetic heterogeneity. Interactions between dabigatran and P-gp-affecting drugs have mainly been studied in Phase I trials in healthy volunteers. Verapamil and amiodarone elevated dabigatran concentrations by 50–60% and clarithromycin by 19%. In a substudy of the RE-LY trial, the influence of proton-pump inhibitors, amiodarone and verapamil on the bioavailability of dabigatran was shown to be significant [10]. Of interest, several of the cases with bleeding complications occurred in patients taking P-gp-affecting drugs (Table 1). Thus, the clinical relevance of drug-interactions with P-gp-affecting drugs and NOAs might be higher than initially expected.

From these findings we conclude that before NOAs may be widely used for stroke prevention more information about the relevance of impaired renal function and drug interactions is warranted. Furthermore, renal function must be monitored diligently in patients treated with NOAs, especially if there is already an tendency for renal impairment.

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