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CLINICAL STUDY

Low testosterone levels predict all-cause mortality and cardiovascular events in women: a prospective cohort study in German primary care patients

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Abstract

Objective: Although associations between testosterone and cardiovascular (CV) morbidity in women have been proposed, no large prospective study has evaluated potential associations between testosterone and mortality in women. The objective was to determine whether baseline testosterone levels in women are associated with future overall or CV morbidity and mortality.
Design: Prospective cohort study with a 4.5-year follow-up period.
Methods: From a representative sample of German primary care practices, 2914 female patients between 18 and 75 years were analyzed for the main outcome measures: CV risk factors, CV diseases, and all-cause mortality.
Results: At baseline, the study population was aged 57.96 ± 14.37 years with a mean body mass index of 26.71 ± 5.17 kg/m^2. No predictive value of total testosterone for incident CV risk factors or CV diseases was observed in logistic regressions. Patients with total testosterone levels in the lowest quintile Q1, however, had a higher risk to die of any cause or to develop a CV event within the follow-up period compared to patients in the collapsed quintiles Q2–Q5 in crude and adjusted Cox regression models (all-cause mortality: Q2–Q5 versus Q1: crude hazard ratios (HR) 0.49, 95% confidence interval (CI) 0.33–0.74; adjusted HR 0.62, 95% CI 0.42–0.939; CV events: Q2–Q5 versus Q1: crude HR 0.54, 95% CI 0.38–0.77; adjusted HR 0.68, 95% CI 0.48–0.97). Kaplan–Meier curves revealed similar data.
Conclusions: Low baseline testosterone in women is associated with increased all-cause mortality and incident CV events independent of traditional risk factors.

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Introduction

Men have a higher risk of cardiovascular (CV) morbidity and mortality than women, and it has been hypothesized that high testosterone levels in women may be a critical factor exerting detrimental effects on the CV system (1). This is supported by findings from cross-sectional and few longitudinal studies, in which positive associations between total and/or bioavailable testosterone and insulin, insulin resistance, overweight, glucose, hemostatic and inflammatory markers, carotid artery intimal-medial thickness, and adverse lipid profiles have been reported (2–7). Also subjects with higher testosterone have higher risk for disease entities such as the metabolic syndrome (MS), type 2 diabetes mellitus (T2DM), and coronary artery disease (CAD) in cross-sectional and case–control studies as well as a few longitudinal studies (8–14).

Other studies did not report any significant relationships or have suggested a positive effect of testosterone on CV diseases in women. Golden, for instance, reported associations with insulin resistance, but did not find any association of increasing quartiles of bioavailable testosterone with T2DM in 1973 postmenopausal women after adjusted analyses (5). Bernini *et al.* (15) reported that higher androgen concentrations in women are related to lower carotid wall thickness, a proxy of carotid artery atherosclerosis, independent of other CV risk factors, and the only published prospective study on endogenous sex hormones and CV disease and ischemic heart disease death in postmenopausal women, the Rancho Bernardo study, reports no

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predictive role of either high or low testosterone on these outcomes during 19 years of follow-up period (16).

In a recent study, Tivesten *et al.* (17) reported a nearly doubled risk of death (hazard ratios (HR) 1.96, 95% confidence interval (CI) 1.46–2.62) in men with low testosterone (quartile 1 versus quartiles 2–4) (17). To our knowledge, our study is the largest prospective study investigating the relationship of testosterone with overall mortality in women.

Subjects and methods

Study population

This study was performed as a prospective cohort study within the DETECT cohort (Diabetes Cardiovascular Risk-Evaluation: Targets and Essential Data for Commitment of Treatment; http://www.detect-studie.de), a large-scale, cross-sectional study with a 4.5-year prospective–longitudinal component (55 518 unselected patients, 59% women and 41% men, of 3188 primary care physicians) as previously described. The DETECT study received the approval of the ethics committee of the Carl Gustav Carus Medical faculty at the Technical University of Dresden (AZ: EK149092003; Date: 16.09.2003), and all patients gave written informed consent. Methods and design were presented in detail elsewhere (18–20). Blood samples were taken from a random subset of these patients ($n=7519$) in 2003 to investigate a wide range of laboratory markers. For the laboratory subsample, a comprehensive 4.5-year follow-up period was conducted in 2007/2008. Testosterone levels were available in a random subsample of 2914 women who were finally included in this analysis.

In general, DETECT study attrition could be kept low (93.9 and 93.1% primary response rates) due to several measures in place such as payments for complete documentation and a systematic patient tracking system to monitor critical outcomes such as hospitalization or mortality. Therefore, information bias due to patient dropout should be low, and misclassification of the hard outcomes such as death should be minimized.

Assessment of CV risk factors, CV diseases, and covariates

Clinical assessments were conducted using i) standardized patient questionnaires, and ii) standardized clinical interview and assessments of physicians with regard to the presence and severity of a wide range of illnesses as previously described (20).

All information was gathered at baseline and at follow-up periods (in 2003, 2004, and 2007/2008).

Physicians were asked to diagnose diabetes mellitus, hyperlipidemia, hypertension, CAD, and other diagnoses using standardized questionnaires as previously described (20). Current medication with a focus on CV treatments was recorded. A total of 23 medications could be marked, including one checkbox for further medication (20).

The behavioral variables such as smoking status (coded as current smoker, ex-smoker, and non-smoker as well as numbers of cigarettes per day), physical activity (coded as less or more than 2 h/week), and alcohol consumption (reported as numbers of drinks per week) were based on patients' interview assessments at baseline as previously described (18, 20, 21).

Physicians were instructed to measure weight, height, blood pressure, and waist circumference (WC) according to a standardized protocol as previously described (21). Systolic (SBP) and diastolic blood pressure (DBP) was measured by indirect cuff sphygmomanometry after several minutes of rest in a sitting position. Use of an appropriate cuff size was advised. WC was measured with a tape measure midway between the lowest rib and the pelvis. The following anthropometric parameters were calculated: body mass index (BMI, weight in kg divided by the square of height in meters) and waist-to-height ratio (WHtR, WC divided by measured height in cm) as previously described (18, 20–22).

According to the guidelines of the WHO (WHO, 2000 EK IV; EASO, 2002 EK IV) and the German guidelines of the Deutsche Adipositas Gesellschaft (DAG), Deutsche Diabetes Gesellschaft (DDG), Deutsche Gesellschaft für Ernährung (DGE), and Deutsche Gesellschaft für Ernährungsmedizin (DGEM), a WHtR of >0.54 was defined as abnormal, indicating visceral obesity.

MS was defined according to the International Diabetes Federation (IDF). For the IDF classification, criterion No. 1 and criteria Nos 2–5 needed to be fulfilled for women: 1, WC ≥ 80 cm; 2, triglycerides >150 mg/dl or intake of lipid-reducing therapy; 3, high-density lipoprotein cholesterol (HDL-C) <50 mg/dl or intake of lipid-reducing therapy; 4, SBP≥ 130 mmHg or DBP≥ 85 mmHg or intake of hypertensive drugs; 5, fasting glucose ≥ 100 mg/dl or type 2 diabetes or intake of oral antidiabetics (www.idf.org/metabolic_syndrome). T2DM was defined as fasting blood glucose level ≥ 126 mg/dl or non-fasting blood glucose ≥ 200 mg/dl or intake of oral antidiabetic drugs.

All measured variables were used as continuous and/or dichotomized variable as appropriate in this study.

Assessment of death

Death was recorded by the primary care physicians. The causes of death were collected, based on the information of the physician and by death certificate if available. Dates were defined by the date of death on the physicians report. Any CV event was defined as

either angina pectoris, myocardial infarction (MI), percutaneous transluminal coronary angioplasty, bypass, surgery, stroke, transient ischemic attack, or CV death.

Laboratory variables

Blood samples were collected between 0800 and 1000 h and shipped by courier at room temperature within 24 h to the central laboratory at the Medical University of Graz (Austria). On arrival in the central laboratory, the samples were centrifuged immediately, and within another 24 h, serum and plasma were stored at −20 °C until further processing. Testosterone was determined in serum with an electrochemiluminescence immunoassay (Elecsys Autoanalyzer 2010, Modular analytics, Roche Diagnostics). The intra- and inter-assay coefficients of variation (CV) were 2.7 and 5.6% respectively. The measuring range of the testosterone assay was between 0.02 and 15 ng/ml (eight patients had testosterone levels of 0.02 ng/ml: none of the subjects had testosterone levels below 0.02 ng/ml).

Immunological methods are commonly used to measure testosterone (13, 14). Our assay has been calibrated against gas chromatography–mass spectrometry (GC–MS) by the producer showing a strong positive correlation ($r=0.997$; documentation Roche). Additionally, we performed a comparison study for testosterone in women in our own laboratory. The correlation between a liquid chromatography–mass spectrometry (LC–MS technique) and the Elecsys Autoanalyzer 2010 was $r=0.83$ (Fig. 1).

IGF1 was determined with an automated chemiluminescence system (Nichols Institute Diagnostics, San Clemente, CA, USA). The maximal intra- and inter-assay CV were 5 and 7% respectively.

Clinical chemical parameters such as albumin as well as cholesterol and triglycerides were determined on a Roche Modular automatic analyzer. Lipoproteins (HDL-C, low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C)) were determined electrophoretically on the HELENA SAS-3/SAS-4 system. HbAlc was determined chromatographically on an ADAMS HA 8160 analyzing system (ARKRAY Inc., Kyoto, Japan). For all the parameters, reagents and secondary standards were used as recommended by the manufacturers. Inter-assay CV of these methods are provided elsewhere (20).

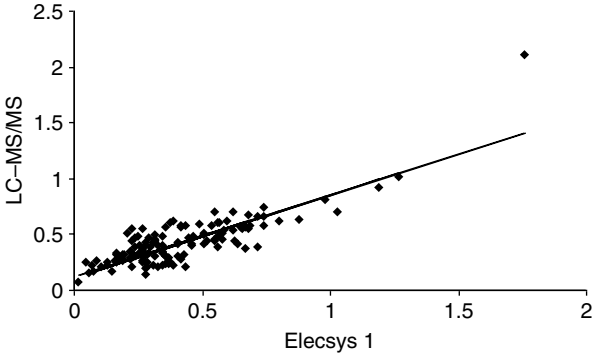

Figure 1 Correlation of Elecsys LC–MS/MS for testosterone in women in DETECT laboratory.

Statistical analysis

Women were stratified according to testosterone quintiles at baseline (testosterone quintiles: Q1, 0.020–0.237 ng/ml; Q2, 0.238–0.343 ng/ml; Q3, 0.344–0.472 ng/ml; Q4, 0.473–0.655 ng/ml; Q5, 0.657–9.340 ng/ml). Quintiles were used for better discrimination given that increased n value allowed us to do so. Further analyses were performed (i) contrasting Q2–Q5 against Q1. At baseline, 11.2% presented with elevated total testosterone levels (total testosterone >0.82 ng/ml as defined by Roche Diagnostics) over all age groups. In premenopausal women 13.25% and in postmenopausal women 9.4% of the patients fulfilled the criteria of hyperandrogenemia.

For descriptive statistics, we performed ANOVA in case of normally distributed continuous variables or χ^2-tests in case of discrete outcomes to compare differences between quintiles (Table 1).

Spearman rank correlations were calculated for the univariate associations between testosterone and CV risk factors (data not shown).

Univariate and multivariate models were applied adjusting in different models for baseline age, BMI, smoking, IGF1 and albumin, menopausal status, physical activity, alcohol use, hypertension, hyperlipidemia, and diabetes mellitus as appropriate (Table 2).

Cox proportional hazards regression models were performed to analyze the relationship between testosterone and all-cause mortality and CV events (Tables 3 and 4). Unadjusted and adjusted (for age, BMI, and smoking) Kaplan–Meier survival curves contrast survival over time between testosterone quintiles Q2–Q5 versus Q1 (Fig. 2).

Data were analyzed using the statistical software SAS (Version 9.2, SAS Institute Inc., Cary, NC, USA). All P values presented are two tailed; $P<0.05$ was considered statistically significant.

This manuscript was written in accordance with the STROBE statement, giving guidelines for reporting observational studies (23).

Results

At baseline, the age of the study population was 57.96 ± 14.37 (mean $\pm$ S.D.) years, with a mean height of 163.6 ± 6.50 cm, a mean weight of 71.49 ± 14.22 kg, and a mean BMI of 26.71 ± 5.17 kg/m^2. Out of 2914 women, 1394 were postmenopausal and 1185 were premenopausal (with unknown status for the remaining 335 women).

Table 1 Patients' characteristics in relation to testosterone quintiles (Q1–Q5) at baseline 2003. Values are expressed as geometric means or numbers. Reported *P* values according to ANOVA or χ^2-tests.

	Testosterone quintiles					
	Q1	Q2	Q3	Q4	Q5	***P* value**
Age						
Age, years (mean)	62.5	60.5	58.1	55.3	53.5	<0.001
Postmenopausal women (*n*)	315	311	284	258	226	<0.001
Anthropometric parameters						
Weight, kg (mean)	70.0	71.7	72.0	71.2	71.9	NS
Height, cm (mean)	162.6	163.3	163.9	164.1	164.2	0.0003
Waist circumference, cm (mean)	90.8	91.6	90.5	89.5	90.0	NS
Hip circumference, cm (mean)	104.3	105.5	105.3	104.2	104.3	NS
WHR (mean)	0.87	0.87	0.86	0.86	0.86	NS
WHtR (mean)	0.56	0.56	0.55	0.55	0.55	0.025
BMI, kg/m^2 (mean)	26.7	27.2	26.9	26.6	26.7	NS
Risk factors						
Systolic blood pressure, mmHg (mean)	133.5	133.6	132.3	131.2	129.7	0.008
Diastolic blood pressure, mmHg (mean)	79.4	79.7	80.1	78.8	79.2	NS
Mean blood pressure, mmHg (mean)	105.9	106.0	105.9	104.6	104.2	NS
Total cholesterol, mg/dl (mean)	230.7	228.7	231.7	222.3	230.7	<0.001
LDL cholesterol, mg/dl (mean)	129.6	128.3	131.4	124.5	126.0	0.009
HDL cholesterol, mg/dl (mean)	60.7	60.6	60.8	60.4	57.9	NS
Triglycerides, mg/dl (mean)	144.9	139.8	133.7	125.9	128.8	0.002
Fasting plasma glucose, mg/dl (mean)	96.2	100.9	101.0	98.8	101.0	NS
HbAlc, % (mean)	5.5	5.6	5.6	5.5	5.5	0.041
Biomarkers						
Albumin, g/dl (mean)	4.4	4.5	4.5	4.5	4.5	<0.001
IGF1, ng/ml (mean)	117.6	120.5	125.1	134.9	140.6	<0.001
Testosterone, ng/ml (mean)	0.16	0.29	0.41	0.55	1.43	<0.001
Behavioral variables						
Physical activity, >2 h/week (*n*)	273	292	283	276	270	NS
Current smoker (*n*)	39	52	71	92	93	<0.001
Cigarets per day (current smokers) (mean)	16.2	13.6	12.1	12.8	13.4	0.03
Current alcohol drinkers (regular/daily) (*n*)	13	20	25	24	13	0.04
Numbers of drinks per week (mean)	2.06	2.09	2.03	2.35	2.26	0.01

Table 1 presents the distribution of patients' characteristics with regard to testosterone levels in quintiles (Q1–Q5). Using ANOVA and χ^2-tests for binary variables, we found that women with low testosterone levels were significantly older and more likely to be postmenopausal. Arithmetic means of anthropometric measures were not significantly different between the quintiles Q1–Q5 besides a higher WHtR in the lower testosterone quintiles.

Significant differences between testosterone quintiles were additionally found for SBP, total cholesterol, LDL-C, triglycerides, HbAlc, albumin, IGF1, as well as alcohol drinking and smoking status. Mean DBP, mean blood pressure, HDL-C, fasting glucose, and physical activity were not differently distributed between testosterone quintiles.

Spearman correlations between testosterone with CV risk factors revealed negative correlations of testosterone with age, menopausal status, waist-to-hip ratio (WHR), WHtR, SBP, mean blood pressure, total cholesterol, HDL-C, triglycerides, HbAlc, and smoking status as per cigarettes per day (data not shown). Significant positive correlations for total testosterone were found for height, albumin, IGF1, and alcohol consumption as per numbers of drinks per week (data not shown). As expected, IGF1 was highly correlated with many of the risk factors under investigation (18).

Total testosterone in relation to CV risk factors and incident CV diseases during the follow-up period

In prospective analyses, we excluded patients with the respective prevalent CV risk factor or disease at baseline as appropriate. Subsequently, we calculated the incidences of CV risk factors (adverse anthropometric measures, hypertension, hyperlipidemia, T2DM, and MS) and CV diseases (CAD, MI, and stroke) during the follow-up period of 4.5 years.

Table 2 reports on the odds ratios (ORs) for newly diagnosed CV risk factors and diseases with respect to baseline testosterone levels stratified in quintiles. In unadjusted models, we observed lower incidences of hypertension in patients with testosterone levels in quintiles Q2, Q3, Q4, and Q5 or collapsed Q2–Q5 compared to Q1 at baseline (P for trend$=0.0370$). The incidence of being overweight was higher in Q2

Table 2 Incident cardiovascular (CV) risk factors and morbidity in relation to baseline testosterone (testosterone quintiles with lowest quintile Q1 as the reference category).

		Q1	Q2			Q3			Q4			Q5			Q2–Q5 versus Q1		
		n (%)	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI
Anthropometric parameters																	
Pathological WHtR	Crude	55 (28.1)	62 (30.4)	1.12	0.73–1.72	52 (23.4)	0.78	0.51–1.22	55 (23.5)	0.79	0.51–1.22	55 (24.8)	0.84	0.55–1.31	224 (25.4)	0.87	0.62–1.23
	Adjusted			1.20	0.72–2.00		0.83	0.49–1.39		1.03	0.62–1.73		1.02	0.60–1.73		1.01	0.67–1.53
Overweight*		2 (25.0)	3 (60.0)	4.50	0.41–49.63	1 (33.3)	1.50	0.08–26.86	3 (60.0)	4.50	0.41–49.63	1 (33.3)	1.50	0.08–26.86	8 (50.0)	3.00	0.46–19.59
Risk factors																	
Hypertension	Crude	79 (26.9)	64 (22.4)	0.78	0.54–1.15	75 (23.0)	0.81	0.56–1.17	60 (19.1)	0.64	0.44–0.94	70 (20.8)	0.72	0.50–1.04	269 (21.3)	0.74	0.55–0.99
	Adjusted			0.93	0.61–1.42		1.05	0.70–1.58		1.14	0.74–1.76		1.31	0.85–2.01		1.09	0.78–1.51
Hyperlipidemia	Crude	81 (24.1)	87 (25.6)	1.08	0.76–1.53	79 (24.0)	0.99	0.70–1.42	73 (21.7)	0.87	0.61–1.25	78 (20.4)	0.81	0.57–1.15	317 (22.8)	0.93	0.70–1.23
	Adjusted			1.12	0.78–1.61		1.18	0.81–1.72		1.06	0.72–1.56		1.05	0.72–1.53		1.10	0.82–1.49
Type 2 diabetes mellitus	Crude	19 (4.4)	27 (6.1)	1.42	0.78–2.60	23 (5.3)	1.24	0.66–2.31	20 (4.6)	1.05	0.55–1.99	19 (4.4)	1.01	0.53–1.93	89 (5.1)	1.18	0.71–1.96
	Adjusted			1.43	0.77–2.67		1.40	0.73–2.67		1.42	0.73–2.77		1.45	0.73–2.85		1.42	0.84–2.40
Metabolic syndrome IDF	Crude	51 (23.0)	67 (29.8)	1.42	0.93–2.17	57 (23.0)	1.00	0.65–1.54	46 (18.4)	0.76	0.48–1.18	49 (20.8)	0.88	0.56–1.37	219 (22.8)	0.99	0.70–1.40
	Adjusted			1.63	1.02–2.61		1.08	0.67–1.74		1.07	0.65–1.77		1.26	0.76–2.06		1.25	0.85–1.84
Cardiovascular morbidity																	
Coronary artery disease	Crude	43 (9.6)	40 (8.6)	0.88	0.56–1.39	40 (8.5)	0.87	0.55–1.36	39 (8.4)	0.86	0.54–1.35	35 (7.4)	0.75	0.47–1.19	154 (8.2)	0.84	0.59–1.19
	Adjusted			0.93	0.58–1.50			0.64–1.68			0.74–1.99		1.09	0.65–1.81			0.72–1.53
Myocardial infarction	Crude	2 (0.4)	4 (0.8)	1.93	0.35–10.60	7 (1.4)	3.40	0.70–16.46	6 (1.2)	2.95	0.59–14.67	1 (0.2)	0.49	0.04–5.39	18 (0.9)	2.19	0.51–9.47
	Adjusted			1.90	0.34–10.49		3.82	0.78–18.61		3.98	0.79–20.09		0.70	0.06–7.86		2.63	0.60–11.45
Stroke	Crude	10 (2.0)	2 (0.4)	0.20	0.04–0.90	9 (1.8)	0.89	0.36–2.22	6 (1.2)	0.59	0.21–1.65	7 (1.4)	0.69	0.26–1.83	24 (1.2)	0.59	0.28–1.25
	Adjusted			0.23	0.05–1.09		1.18	0.46–3.03		0.93	0.32–2.67		0.91	0.32–2.62		0.78	0.36–1.72

Adjusted for age, BMI and smoking. *BMI > 25 kg/m^2.

and Q4 compared to Q1 and in collapsed quintiles Q2–Q5 versus Q1 (P for trend = 0.0055). However, further adjustments abolished these effects indicating a strong confounding effect of age and BMI for hypertension.

Additional analyses adjusting for the presence of disease at baseline rather than exclusion of patients with prevalent diseases were largely confirmatory of the analyses presented herein.

When analyzing the predictive value of testosterone levels for incident CV morbidity (CAD, MI, and stroke), crude analyses and further adjustments (for age, BMI or smoking or age, BMI, smoking, physical activity, IGF1, and albumin and where applicable diabetes, hypertension, and hyperlipidemia) led to non-significant results of all three investigated outcomes (CAD, MI, and stroke) (Table 2). Additional analyses adjusting for baseline diseases rather than exclusion of patients with prevalent diseases were largely confirmatory of the analyses presented herein.

Total testosterone in relation to all-cause mortality and CV events during the follow-up period (HR)

To evaluate independent associations of total testosterone with all-cause mortality (Table 3) and any CV events (Table 4) before and after adjusting for age, BMI, and smoking, and for age, menopausal status, BMI, WHR, SBP, LDL-C, HDL-C, triglycerides, HbAlc, albumin, alcohol, and smoking, and for age, menopausal status, BMI, WHR, SBP, LDL-C, HDL-C, triglycerides, HbAlc, albumin, alcohol, smoking, and antihypertensive treatment, we performed Cox proportional hazard regressions.

Patients with testosterone levels in the lowest quintile Q1 (testosterone <0.237 ng/ml) had a higher risk of death from any cause within the follow-up period of 4.5 years compared with patients in Q3, Q4, or Q5 and the collapsed quintiles Q2–Q5 in crude and adjusted models (Q2–Q5 versus Q1: crude HR 0.49, 95% CI 0.33–0.74; adjusted HR 0.62, 95% CI 0.42–0.939) (Table 3).

Similar results were found for CV events in crude and adjusted models (Q2–Q5 versus Q1: crude HR 0.54, 95% CI 0.38–0.77; adjusted HR 0.68, 95% CI 0.48–0.97) (Table 4).

In sensitivity analyses, these results were also confirmed collapsing Q3–Q5 contrasted against Q1 or Q1 + Q2 (data not shown).

We further investigated the importance of prevalent diseases on the association between testosterone and mortality as previously described (22). Therefore, we excluded women with prevalence of diabetes mellitus ($n=455$), CV diseases ($n=276$), and cancer ($n=97$) at baseline.

The adjusted HR for all-cause mortality and CV events remain significantly lower for patients in quintiles Q2–Q5 compared with women in Q1 if patients with diabetes mellitus ($P=0.03$ and 0.016 respectively)

Table 3 Baseline testosterone levels in relation to all-cause mortality before and after exclusion of prevalent diseases.

	Q1	Q2			Q3			Q4			Q5			Q2–Q5		
All-cause mortality	*n* (%)	*n* (%)	HR	95% CI	*n* (%)	HR	95% CI	*n* (%)	HR	95% CI	*n* (%)	HR	95% CI	*n* (%)	HR	95% CI
Crude analysis	35 (6.8)	21 (4.1)	0.59	0.34–1.01	18 (3.5)	0.50	0.28–0.89	15 (2.9)	0.42	0.23–0.77	16 (3.1)	0.45	0.25–0.81	70 (3.4)	0.49	0.33–0.74
Adjusted	35 (6.8)	21 (4.1)	0.62	0.37–1.07	18 (3.5)	0.59	0.33–1.03	15 (2.9)	0.64	0.35–1.15	16 (3.1)	0.66	0.37–1.19	70 (3.4)	0.62	0.42–0.93
Exclusion of prevalent diabetes at baseline[a]	26 (6.0)	18 (4.1)	0.69	0.38–1.24	12 (2.8)	0.54	0.27–1.06	10 (2.3)	0.54	0.26–1.10	10 (2.3)	0.59	0.28–1.21	50 (2.9)	0.59	0.37–0.95
Exclusion of prevalent CVD at baseline[b]	23 (5.2)	14 (3.0)	0.62	0.32–1.19	11 (2.3)	0.52	0.25–1.07	11 (2.4)	0.67	0.33–1.36	14 (2.9)	0.84	0.43–1.65	50 (2.7)	0.65	0.40–1.06
Exclusion of prevalent cancer at baseline[c]	34 (6.8)	18 (3.7)	0.56	0.32–0.99	16 (3.2)	0.52	0.29–0.94	14 (2.8)	0.62	0.34–1.14	14 (2.8)	0.58	0.31–1.08	62 (3.1)	0.57	0.38–0.85

[a] $n=455$ patients excluded.
[b] $n=276$ patients excluded.
[c] $n=97$ patients excluded.

and cancer ($P=0.007$ and 0.038 respectively) were excluded (Tables 3 and 4). However, when CAD at baseline was excluded, significance for both all-cause mortality and CV events was reduced to a marginal level ($P=0.087$ and 0.076 respectively).

In additional sensitivity analyses, we excluded female patients on oral contraceptives or postmenopausal hormone therapy and hyperandrogenemia including interaction models with similar outcomes as described.

Figure 2 presents the cumulative survival curves for subjects in the testosterone lowest quintiles Q1 with higher all-cause mortality and CV events compared to female patients in quintiles Q2–Q5 (Fig. 2).

Discussion

In this large prospective cohort study, we investigated the predictive value of total testosterone on the incidence of CV risk factors, CV diseases, and mortality in female primary care patients in Germany.

The main finding of our study was the observation that all-cause mortality and CV events in female patients with low testosterone levels (Q1, testosterone <0.237 ng/ml) are elevated compared with patients with testosterone levels in quintiles Q2–Q5, and that this association is largely independent of traditional risk factors. However, no significant prospective associations were found in unadjusted and adjusted analyses in relation to new onset, incident, CV risk factors and CV diseases, implying that, if any, the main association of low testosterone could be not so much with disease occurrence but with disease progression, severity, and/or outcome. Our observational study, albeit prospective and incorporate the time sequence criterion for causality, cannot prove causality. Two hypotheses might explain our findings: i) low testosterone in women may cause or worsen disease and therefore leads to increased mortality rates or ii) low testosterone is simply a marker of disease or poor health.

The first hypothesis may be supported by prior clinical observations in men. Several epidemiological studies in men have demonstrated that low testosterone is associated with higher prevalence and incidence of CV risk factors, CV diseases, and mortality (17, 24, 25). This phenomenon could be mediated through adverse direct and indirect effects of low testosterone on vascular tone, lipid and glucose metabolism, and the hemostatic system (25). In women, however, consequences of low testosterone or androgen deficiency on CV risk factors and diseases are poorly studied. Miller (26), for instance, reviewed that physiological testosterone replacement in women shows a positive effect on the restoration of a normal body composition, however, not consistently (27).

The second hypothesis is that low testosterone is a marker of poor health or more severe disease. This hypothesis is supported by pathological findings that

Table 4 Baseline testosterone levels in relation to cardiovascular events before and after exclusion of prevalent diseases.

	Q1	Q2			Q3			Q4			Q5			Q2–Q5		
Any cardiovascular event	*n* (%)	*n* (%)	HR	95% CI	*n* (%)	HR	95% CI	*n* (%)	HR	95% CI	*n* (%)	HR	95% CI	*n* (%)	HR	95% CI
Crude analysis	45 (10.1)	24	0.50	0.31–0.83	31 (6.7)	0.65	0.41–1.02	28 (6.0)	0.58	0.36–0.94	21 (4.5)	0.43	0.26–0.72	104 (5.6)	0.54	0.38–0.77
Adjusted	45 (10.1)	24	0.52	0.32–0.87	31 (6.7)	0.77	0.49–1.23	28 (6.0)	0.84	0.53–1.35	21 (4.5)	0.64	0.38–1.07	104 (5.6)	0.68	0.48–0.97
Exclusion of prevalent diabetes at baseline[a]	35 (9.1)	20	0.56	0.32–0.97	19 (4.8)	0.63	0.36–1.11	17 (4.2)	0.68	0.38–1.20	13 (3.2)	0.57	0.30–1.08	69 (4.3)	0.60	0.40–0.91
Exclusion of prevalent CVD at baseline[b]	41 (9.5)	21	0.50	0.30–0.86	29 (6.4)	0.80	0.49–1.29	28 (6.1)	0.92	0.57–1.49	21 (4.5)	0.71	0.42–1.21	99 (5.4)	0.72	0.50–1.04
Exclusion of prevalent cancer at baseline[c]	44 (10.2)	23	0.53	0.32–0.89	30 (6.7)	0.76	0.48–1.22	28 (6.2)	0.87	0.55–1.40	20 (4.4)	0.61	0.36–1.04	101 (5.6)	0.69	0.48–0.98

[a] $n=455$ patients excluded.
[b] $n=276$ patients excluded.
[c] $n=97$ patients excluded.

acute and chronic illness may decrease testosterone levels, which is, again, more comprehensively studied in men (28). It has been hypothesized that the stress following acute or chronic diseases with subsequent hypercortisolism may exert direct inhibitory effects on the hypothalamic–pituitary–gonadal system, resulting in hypogonadism. In women, it is known that a state of hypogonadotropic hypogonadism is not only associated with reduced estrogen, but also associated with lower testosterone levels (29).

Mohamad *et al.* (30), Dobrzycki *et al.* (31), Pugh *et al.* (32), and other authors reported that in men, specifically, CV diseases such as CAD are associated with hypoandrogenemia, but the results for women are less well studied and/or conclusive (30–34).

Another argument for the described hypothesis is supported by our results presented in Table 4. The exclusion of patients with prevalent CV diseases and prevalent CV disease or diabetes mellitus or cancer (but not prevalent diabetes mellitus alone or cancer alone) attenuated the statistical significance for all-cause mortality and CV events. We can therefore hypothesize that low testosterone is not only a marker of poor health, in general, but also more specifically and perhaps mainly of CV morbidity and mortality.

Other than stress, underlying diseases causing low testosterone levels in women are oophorectomy, adrenal insufficiency, supraphysiologic glucocorticoid administration, GnRH administration, anorexia nervosa, or AIDS wasting. Although the prevalence of patients with these diseases in our cohort is apparently extremely low, nevertheless they might have contributed to higher death rates due to the underlying disease independently of low testosterone levels.

To our knowledge, this is the most comprehensive study on this topic in a sample that includes both pre- and postmenopausal women. Only one smaller scale epidemiological study in 651 postmenopausal women investigated mortality over 19 years of follow-up period in relation to testosterone levels (16). The authors' comparisons of baseline total and bioavailable testosterone levels between female patients who died from CV disease and those who did not revealed lower baseline testosterone levels in the first group compared with the second group, which would be in line with our results. Differences with respect to significance of HR among this and our study could be due to differences in the two population samples (with both pre- and postmenopausal women included in our cohort with a lower mean age (57.9 versus 66.7 years)) and higher patient numbers ($n=2914$ versus $n=651$) despite fewer years of follow-up period, which might have raised the power. Additionally, a very recent paper by Laughlin *et al.* (14) reported that low levels of testosterone are associated with an increased risk of coronary heart disease (CHD) events prospectively, which additionally underlines our findings (14).

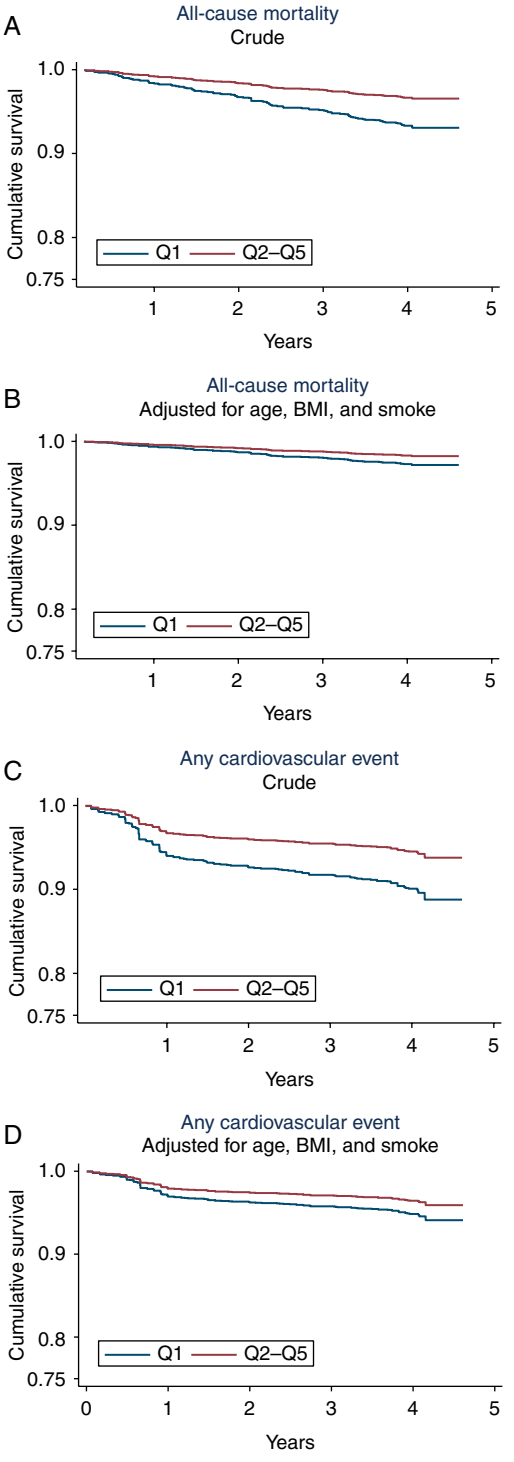

Figure 2 Unadjusted and adjusted (for age, BMI, and smoking) Kaplan–Meier curves for all-cause mortality (A and B) and any cardiovascular event (C and D). The graphs represent the cumulative survival of women in the lowest testosterone quintile Q1 compared to quintiles Q2–Q5.

Strengths and limitations

Testosterone measurements were performed using an electrochemiluminescence immunoassay (Elecsys system, Roche). This technique has been used as the standard to date and has been calibrated against the GC–MS technique showing a strong positive correlation. Ding *et al.* (8), Patel *et al.* (13), and Laughlin *et al.* (14) recently reported reliable results on testosterone in women with the immunoassay-based technique.

Our results are based on a single testosterone measurement. Despite circadian and other variability of testosterone levels, prior studies have demonstrated that intra-individual variance of total testosterone is small and similar in female patients with hyperandrogenemia or normal testosterone levels, and the authors propose that single androgen measurements are legitimate means to determine androgen status (35). Moreover, any misclassification would be random and would have suppressed effect estimates toward the null. Not taking the time of the menstrual cycle for premenopausal women into account might even further underestimate true relationships. We neither studied the role of free testosterone, SHBG, or estradiol nor their possible interactions with total testosterone. This is noteworthy, since independent associations of low SHBG, for instance, with the MS and CV risk factors in women with PCOS have been reported (36–38). On the other hand, previous studies found similar risks for total and free testosterone levels and CV risk factors in women.

To minimize measurement errors of the outcome and covariates, data checks for plausibility and completeness were implemented at data entry to further guarantee data quality. Potential confounders were addressed by adjustments for traditional clinical risk factors. Time differences occurred in the follow-up period between individual subjects and could have created non-differential misclassification, which from the statistical point of view would have resulted in a suppression of effect estimates and loss of statistical significance. However, the fact that our results are significant indicates that our methods and statements should be valid.

To avoid confounding by those patients with extremely elevated testosterone levels, possibly associated with pathological conditions such as androgen-producing ovarian and adrenal neoplasms, Cushing's syndrome, polycystic ovary syndrome, and the intake of exogenous androgens, we repeated all analyses excluding those patients with pathological testosterone values above 0.82 or >0.59 ng/ml (according to the Rotterdam criteria Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group). In the second step, we excluded all patients on hormonal substitution. In addition to age, we also stratified and adjusted for menopausal status (including interaction models). Since we observed similar results, we decided to report on all women in DETECT.

Although a stratified sampling scheme was used to recruit the patients in DETECT, the population under investigation reflects a primary care sample and the results may not be generalizable to the general German population.

Additionally, our study cohort consists of mainly relatively older women. The results and conclusions may therefore not be generalizable to a younger female population. Why we did not observe significant results for incident CV risk factors and diseases despite significant results for the Cox regression analysis on mortality needs to be discussed. We excluded respective prevalent risk factors and diseases in the logistic regression analyses (Table 2), which might have led to relatively low cases for the follow-up period and might have underpowered the study for these outcomes. On the other hand, it is conceivable that testosterone is rather a marker of progression to and/or indicator of serious disease and death than of the simple occurrence of CV diseases, which would be consistent with our second hypothesis as discussed above.

In conclusion, this study shows in a large primary care cohort that low baseline testosterone in relatively older women is associated with increased all-cause mortality and CV events, which is largely independent of traditional risk factors. Our results support the notion that the hormonal status of relatively older women might have consequences in relation to CV risk and the diagnostic work-up in women at risk for CV disease. Future studies are needed to confirm these results, to explore underlying mechanisms, and to determine whether this finding may prove to have direct implications on the clinical management of these patients.

Declaration of interest

There are no conflicts of interest for C Sievers, J Klotsche, L Pieper, H J Schneider, G K Stalla, and C Mantzoros. H U Wittchen and W März received an unrestricted educational grant for DETECT from Pfizer and additional fees for consulting.

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