Endogenous Testosterone, Endothelial Dysfunction, and Cardiovascular Events in Men with Nondialysis Chronic Kidney Disease

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Summary

Background and objectives Deterioration of kidney function impairs testosterone production, with hypogonadism being common in men with chronic kidney disease (CKD). In nonrenal populations, testosterone is suggested to participate in the atherosclerotic process. In male dialysis patients, we showed that low testosterone increases the risk of mortality. We here studied plausible links among testosterone levels, vascular derangements, and cardiovascular events in nondialysis CKD men.

Design, setting, participants, & methods This was a cross-sectional analysis in which flow-mediated dilation (FMD) was assessed in 239 CKD male patients (stages 1 to 5; mean age 52 ± 12 years), together with routine measurements, serum total and free testosterone, and follow-up for cardiovascular outcomes.

Results Total and free testosterone levels decreased in parallel with the reduction of kidney function. Multiple regression analyses showed that total and free testosterone significantly and independently contributed to explain the variance of FMD. After a median follow-up of 31 months (range 8 to 35 months), 22 fatal and 50 nonfatal cardiovascular events occurred. In Cox analysis, the risk of cardiovascular events was reduced by 22% for each nanomole-per-liter increment of total testosterone. This reduced risk persisted after adjustment for age, renal function, diabetes mellitus, previous cardiovascular history, C-reactive protein, albumin, and FMD. The same was true for free testosterone concentrations.

Conclusions The reduction in endogenous testosterone levels observed with progressive CKD was inversely associated with endothelial dysfunction and exacerbated the risk of future cardiovascular events in nondialysis male CKD patients.

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Introduction

Chronic kidney disease (CKD) *per se* is associated with a wide range of metabolic alterations, including disorders in the secretion of hormones and the response of target tissues, causing several endocrine dysfunctions that may contribute to worse outcomes (1,2). Among those, hypogonadism (*i.e.*, testosterone deficiency) is the most common gonadal alteration in men, mainly because of reduced prolactin clearance (3) and uremic inhibition of luteinizing hormone signaling at the level of the Leydig cells (4).

In the general population, a growing body of evidence suggests that testosterone deficiency may contribute to the onset, progression, or both of cardiovascular disease (CVD) (5). Low testosterone levels in apparently healthy male populations have been identified as a predisposing risk factor to increased mortality and cardiovascular comorbidity (6). It has recently been reported that low endogenous testosterone values were also associated with increased risk of death in male hemodialysis patients (7,8). Interestingly, testosterone deficiency in men undergoing hemodialysis has also been related to arterial thickening, atherosclerotic plaque occurrence, and reduced flow-mediated dilation (FMD) (9). The high prevalence and amenability to intervention makes hypogonadism an attractive potential therapeutic target in CKD men, meriting further investigation.

Progression toward ESRD conveys a worsening of endothelial function and exposes patients to increased risk of developing premature vascular disease and cardiovascular morbidity (10–12). At present, the prevalence of male hypogonadism or the effect of endogenous testosterone on vascular derangements or future cardiovascular outcomes in nondialysis CKD stages is unknown. Against this background, we investigated plausible associations among total and free testosterone concentration, FMD, and cardio-

vascular risk *post hoc* in a cohort of 239 etiologically diagnosed nondialysis CKD men with FMD assessments and prospective follow-up for cardiovascular events.

Materials and Methods

Patients

The ethical committee of the Gülhane School of Medicine (Etlik-Ankara, Turkey) approved the study, and informed consent was obtained from each subject. Subjects were prevalent patients recruited between March 2006 and June 2010, and the protocol has been published elsewhere in more detail (13). During this period, 817 patients were referred to the Renal Unit of the Gülhane School of Medicine Medical Center in Ankara, Turkey, for the first time because of suspected or manifest renal failure. All patients were diagnosed as having CKD according to their estimated GFR (eGFR) and the presence of kidney injury as defined by National Kidney Foundation Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines. By protocol, and to minimize any confounding effects of conditions that may influence endothelial dysfunction, 248 patients who were taking drugs that may influence endothelial function were excluded, including angiotensin converting enzyme inhibitors (ACEIs; $n = 96$), angiotensin receptor blockers (ARBs; $n = 79$), statins ($n = 24$), erythropoietin ($n = 11$), or supplemental vitamin pills ($n = 38$). In the design of this cohort, calcium channel antagonists and β-blocker agents were assumed to have reduced effect (or at least a lesser evidence) on endothelial function. Otherwise, exclusion criteria including acute infections and unwillingness to participate in the study were applied ($n = 33$). Fifty-three eligible patients dropped out during observation for the following reasons: lost contact or transferred to other dialysis units ($n = 17$), viral hepatitis ($n = 9$), vasculitis ($n = 5$), and refusal to participate ($n = 22$). Female patients were excluded from this analysis ($n = 244$), leaving 239 male patients with a mean age of 54 ± 12 years in the study.

Thirty-eight of the patients were on antihypertensive therapy (21 patients were treated with calcium channel antagonists, 5 with β-blocker agents, 3 with α-blockers, and 9 with loop diuretics; no patients were on nebivolol). Fifty-three of the patients were on antidiabetic therapy (21 patients were treated with oral antidiabetics and 32 with insulin). Once baseline determinations were performed, drugs were prescribed when necessary upon physician's judgment and following common practice. As such, as soon as diabetic nephropathy was diagnosed, all patients with oral antidiabetics were changed to insulin. No patients were on testosterone or androgen replacement medication. Seventy-nine patients (33%) had a history of CVD as defined by medical history and/or clinical findings at the time of enrollment. Of these 79 patients, 27 had suffered from cerebrovascular disease (stroke), 36 from CVD (acute myocardial infarction, angina pectoris, or had undergone coronary artery bypass surgery); 11 had a history of peripheral ischemic atherosclerotic vascular disease and 5 patients had a history of an aortic aneurysm. Smoking habits were recorded as follows: 107 patients were former or current smokers and 132 were nonsmokers.

Patients were classified with respect to eGFR levels from stage 1 to 5 as determined by K/DOQI (Table 1), which was calculated according to the simplified version of the Modification of Diet in Renal Disease formula as defined by Levey *et al.* (14) ($GFR = 186 \times Pcr^{-1.154} \times age^{-0.203} \times 1.212$ [*if black*], where *Pcr* is the plasma creatinine concentration). Additionally, patients were followed for time-to-event analysis of cardiovascular outcomes, until cardiovascular event, or until death, whichever came first.

Laboratory Measurements

All samples were obtained in the morning after overnight fasting. Routine biochemical measurements included serum albumin, hemoglobin, parathyroid hormone (PTH), calcium, phosphate, total cholesterol, and triglycerides. For the measurement of high-sensitivity C-reactive protein (CRP), serum samples were diluted at a ratio of 1:101 with the diluent solution. Calibrators, kit controls, and serum samples were all added to each microwell with an incubation period of 30 minutes. After three washing intervals, 100 μl of enzyme conjugate (peroxidase-labeled anti-CRP) was added to each microwell for an additional 15-minute incubation at room temperature in the dark. The reaction was stopped with a stop solution and photometric measurement was performed at a wavelength of 450 nm. The amount of serum samples was calculated as milligrams per liter with a graphic that was made by noting the absorbance levels of the calibrators. Serum total testosterone levels were quantified in a Modular Analytics E170 Module (Roche Diagnostics, Indianapolis, IN) using a RIA kit (Diagnostic Systems Laboratories, Webster, TX) and counted by a gamma counter (multi crystal-Berthold LB 2111, Germany) to determine serum free testosterone levels. The RIA kit for the active free testosterone coated tube (Diagnostic Systems Laboratories, Webster, TX) was a solid-phase ^{125}I RIA. Routine assay was based on the direct immunoassay principle (analog-free testosterone). The latter makes use of a labeled testosterone analog that competes with serum free testosterone for binding to a testosterone-specific antiserum immobilized to a propylene tube. The amount of ^{125}I-labeled testosterone analog bound to antibody is inversely proportional to the concentration of the free testosterone present. The separation of free and bound antigen is achieved by decanting or aspirating the antibody-coated tubes. The reference ranges for total and free testosterone concentrations according to the kit manufacturers were 10 to 28 nmol/L (or 288 to 800 ng/dl) and 8.6 to 54.6 pg/ml, respectively.

Vascular Assessments

Arterial BP was measured by a physician in the morning 3 consecutive times after a 15-minute resting period, and mean values were calculated for systolic and diastolic pressure in all patients.

Endothelium-dependent vasodilation (FMD) and endothelium-independent vasodilation (NMD) of the brachial artery were assessed noninvasively using high-resolution ultrasound. Measurements were made by a single observer using an ATL 5000 ultrasound system (Advanced Technology Laboratories, Inc., Bothell, WA) with a 12-MHz probe. The subjects remained at rest in the supine position for at least 15 minutes before the examination started. The subject's arm was comfortably immobilized in the extended position to

Table 1. Demographic and clinical characteristics of CKD male patients included in the study according to the K/DOQI CKD stage classification

Characteristics	eGFR (ml/min per 1.73 m^2)					*P*
	Stage 1 (≥90) (*n* = 48)	Stage 2 (60 to 89) (*n* = 48)	Stage 3 (30 to 59) (*n* = 47)	Stage 4 (15 to 29) (*n* = 45)	Stage 5 (<15) (*n* = 51)	
Age (years)	53 (30 to 73)	55 (32 to 71)	53 (31 to 73)	56 (33 to 70)	50 (30 to 71)	0.89
Previous CVD (%)	25	35	32	38	35	0.74
Diabetes (%)	19	25	21	27	20	0.78
BMI (kg/m^2)	26.6 ± 2.3	26.4 ± 3.1	25.7 ± 2.6	26.0 ± 3.1	25.2 ± 2.7	0.06
Systolic BP (mmHg)	133 (110 to 157)	134 (119 to 160)	135 (110 to 155)	134 (113 to 170)	135 (110 to 180)	0.33
Diastolic BP (mmHg)	82 (71 to 93)	82 (75 to 92)	86 (80 to 95)	85 (71 to 93)	83 (71 to 97)	0.06
Smoking (%)	46	39	51	52	45	0.82
eGFR (ml/min)	96 (91 to 108)	78 (61 to 89)	48 (31 to 58)	24 (15 to 29)	11 (5 to 14)	<0.001
24-hour proteinuria (g/day)	1.39 ± 0.53	1.33 ± 0.47	1.69 ± 0.78	1.74 ± 0.75	2.35 ± 1.32	<0.001
Hemoglobin (g/dl)	12.6 ± 2.7	12.7 ± 1.9	11.1 ± 2.3	11.2 ± 2.1	10.6 ± 1.8	<0.001
Serum albumin (g/dl)	4.0 (3.5 to 4.6)	4.0 (3.5 to 4.6)	4.1 (3.4 to 4.6)	4.0 (3.5 to 4.6)	3.8 (3.2 to 4.5)	<0.001
CRP (mg/l)	7.3 (3.2 to 11.6)	10.0 (5.0 to 13.0)	16.0 (5.0 to 32.0)	21.0 (4.7 to 33.0)	26.0 (4.0 to 39.0)	<0.001
Serum calcium (mg/dl)	8.7 (8.1 to 9.9)	8.8 (8.0 to 9.8)	8.5 (7.3 to 9.2)	8.2 (7.2 to 9.0)	8.1 (7.2 to 9.0)	<0.001
Serum phosphate (mg/dl)	4.21 ± 0.50	4.19 ± 0.77	4.71 ± 0.83	5.68 ± 1.25	7.13 ± 1.59	<0.001
PTH (pg/ml)	47 ± 11	61 ± 23	130 ± 24	153 ± 21	280 ± 69	<0.001
Flow-mediated dilation (%)	8.4 (7.2 to 9.7)	7.2 (6.0 to 8.3)	6.8 (5.3 to 8.2)	6.2 (5.0 to 8.1)	5.2 (4.0 to 7.2)	<0.001
Nonflow-mediated dilation (%)	12.9 ± 0.5	13.1 ± 0.3	12.9 ± 0.5	13.0 ± 0.4	11.8 ± 0.9	<0.001
Total testosterone (nmol/L)	14.6 (3.0 to 28.1)	13.1 (4.1 to 27.7)	11.9 (2.3 to 22.9)	10.9 (1.9 to 19.2)	8.4 (0.7 to 24.6)	<0.001
Free testosterone (pg/ml)	33.1 (9.1 to 70.0)	39.1 (7.3 to 73.6)	32.0 (1.5 to 66.7)	25.3 (2.8 to 80.5)	18.4 (1.2 to 71.3)	<0.001

CKD, chronic kidney disease; K/DOQI, Kidney Disease Outcomes Quality Initiative; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; BMI, body mass index; CRP, C-reactive protein; PTH, parathyroid hormone.

allow consistent recording of the brachial artery 2 to 4 cm above the antecubital fossa. Three adjacent measurements of end-diastolic brachial artery diameter were made from single two-dimensional frames. All ultrasound images were recorded on S-VHS videotape for subsequent blinded analysis. A pneumatic tourniquet was inflated to 200 mmHg with obliteration of the radial pulse. After 5 minutes, the cuff was deflated. Flow measurements were made 60 seconds postdeflation. After a further 15 minutes, measurements were repeated and again 3 minutes after administration of sublingual glyceryl trinitrate 400 μg. The maximum FMD and NMD dilation diameters were calculated as the average of the three consecutive maximum diameter measurements. The FMD and NMD were then calculated as the percent change in diameter compared with baseline resting diameters. FMD measurements were performed ideally on the same day or within 7 days from blood extraction and biochemical assessment.

Statistical Analyses

All of the statistical analyses were performed using the Stata version 11.1 (Stata Corporation, College Station, TX) statistical package. Non-normally distributed variables were expressed as median (range) and normally distributed variables were expressed as mean ± SD, as appropriate. $P < 0.05$ was considered to be statistically significant. Between-group comparisons were assessed for nominal variables with the χ^2 test and by Kruskal–Wallis test. Spearman's rank correlation was used to determine correlations between variables. Multivariate regression analysis was used to assess the predictors for FMD and serum phosphate levels. Time-to-event analysis of cardiovascular outcomes was done using the Cox proportional hazards model, including adjustment for potential confounding factors. The proportionality assumptions were checked through inspection of the log of the incidence rates. Data are presented in the form of hazard ratios (HRs) and 95% confidence intervals (CIs).

Results

General Characteristics and Categorical Comparisons of Hypogonadism

The demographic and clinical characteristics of the five different CKD stages are given in Table 1. There were no statistically significant differences among the different CKD stages with regards to age, body mass index, diabetes, or history of CVD. Biochemical and vascular assessments are also given in Table 1. Serum albumin, calcium, FMD, and total and free testosterone levels gradually decreased across increasing CKD stages, whereas serum phosphate, PTH, and CRP values increased. Box plots showing the reduction of total and free testosterone levels in parallel with the reduction in eGFR are given in Figure 1. The prevalence of hypogonadism according to the cutoff of 10 nmol/L for total testosterone is given in Figure 2. However, the prevalence of hypogonadism, according to the cutoff of 50 pg/ml for free testosterone, was higher. According to this definition the overall prevalence of hypogonadism was 80%, ranging from 75% in CKD stage 1 patients to 92% in CKD stage 5 patients.

Table 2 depicts patient characteristics according to the presence of hypogonadism. Hypogonadal patients were more often diabetic and with a previous history of CVD. eGFR, calcium, and free testosterone were significantly lower, whereas systolic BP, CRP, phosphate, and PTH were significantly higher. FMD was decreased.

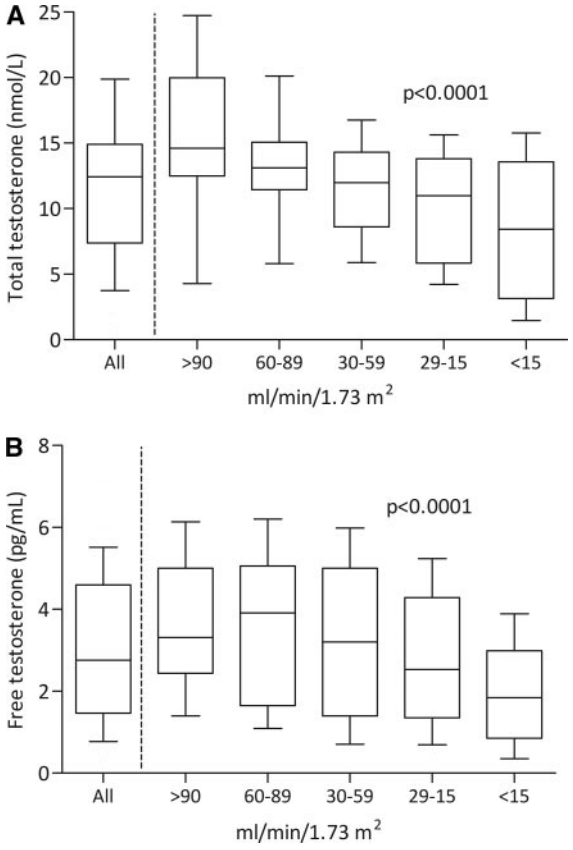

Figure 1. | Box plots depicting (A) total testosterone and (B) free testosterone levels in all studied patients and stratified by levels of estimated GFR.

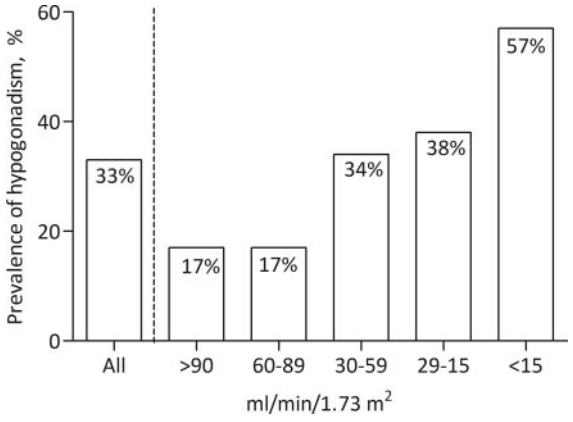

Figure 2. | Prevalence of hypogonadism (defined as total testosterone ≤ 10 nmol/L) in all studied patients and stratified by chronic kidney disease stages.

Multivariate Regressions among Testosterone, Phosphate, and FMD

In univariate analysis, decreased FMD (Spearman's ρ 0.53, $P < 0.0001$, Figure 2) and increased serum phosphate levels

Table 2. Patient characteristics categorized according to the presence of hypogonadism and correlates of total testosterone in CKD male patients

Characteristics	Eugonadal (n = 161)	Hypogonadal (n = 78)	P
Age (years)	54 (30 to 73)	52 (30 to 73)	0.61
History of CVD (%)	27	44	0.01
Diabetes (%)	16	36	<0.001
BMI (kg/m^2)	26.1 ± 2.6	25.7 ± 2.9	0.43
Systolic BP (mmHg)	133 (110 to 157)	135 (113 to 185)	0.002
Diastolic BP (mmHg)	83 (71 to 93)	84 (71 to 97)	0.12
Smoking (%)	45.9	42.3	0.63
eGFR (ml/min)	58 (5 to 106)	23 (7 to 99)	<0.001
24-hour proteinuria (g/day)	1.60 ± 0.78	1.89 ± 1.10	0.19
Hemoglobin (g/dl)	11.7 (7.0 to 16.8)	11.0 (7.0 to 16.9)	<0.001
Serum albumin (g/dl)	4.0 (3.2 to 4.8)	4.0 (3.4 to 4.8)	0.67
CRP (mg/l)	11.2 (3.2 to 37.0)	18.3 (4.0 to 39.0)	<0.001
Serum calcium (mg/dl)	8.5 (7.1 to 10.0)	8.25 (7.0 to 9.7)	0.005
Serum phosphate (mg/dl)	4.8 ± 1.3	5.8 ± 1.7	<0.001
PTH (pg/ml)	113 ± 77	179 ± 105	<0.001
Flow-mediated dilation (%)	7.2 (4.6 to 9.7)	6.2 (4.0 to 8.2)	<0.001
Nonflow-mediated dilation (%)	13.0 (12.5 to 13.2)	12.8 (12.0 to 13.0)	0.02
Total testosterone (nmol/L)	13.8 (12.4 to 16.3)	5.4 (3.1 to 7.3)	–
Free testosterone (pg/ml)	32.5 (33.3 to 72.6)	14.2 (1.23 to 80.5)	<0.001

Hypogonadism was defined on the basis of total testosterone < 10 nmol/L. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; BMI, body mass index; CRP, C-reactive protein; PTH, parathyroid hormone.

(Spearman's ρ −0.34, $P < 0.0001$) strongly correlated with testosterone. We then studied these associations through multivariate analyses. To analyze the independent contribution of testosterone to the variance of phosphate concentrations (Table 3A), we performed a series of multiple regression models. Adjustment for age, eGFR, and serum calcium did not affect the significant association between testosterone and phosphate, but further adjustment for PTH values made this association disappear. The same was true for free testosterone.

To analyze the independent contribution of testosterone to the variance of FMD (Table 4A), we constructed a series of multiple regression models on the basis of traditional and nontraditional risk factors affecting this variable. Adjustment for traditional risk factors (model 1: age, body mass index, total cholesterol, diabetes, previous CVD, sys-

Table 3A. Multiple regression models for serum phosphate (mg/dl) in CKD male patients: Total testosterone

	Unadjusted (β, P) (r^2 = 0.12)	Model 1 (β, P) (r^2 = 0.46)	Model 2 (β, P) (r^2 = 0.63)
Total testosterone (nmol/L)	−0.35 (<0.001)	−0.10 (0.04)	−0.03 (0.54)
Age (years)		0.01 (0.88)	0.02 (0.59)
eGFR (ml/min per 1.73 m^2)		−0.52 (<0.001)	−0.15 (0.07)
Serum calcium (mg/dl)		−0.17 (0.005)	−0.22 (<0.001)
PTH (pg/ml)			0.79 (<0.001)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone.

Table 3B. Multiple regression models for serum phosphate (mg/dl) in CKD male patients: Free testosterone

	Unadjusted (β, P) (r^2 = 0.10)	Model 1 (β, P) (r^2 = 0.47)	Model 2 (β, P) (r^2 = 0.64)
Free testosterone (10 pg/ml)	−0.32 (<0.001)	−0.13 (0.007)	−0.04 (0.32)
Age (years)		−0.01 (0.82)	0.02 (0.57)
eGFR (ml/min per 1.73 m^2)		−0.52 (<0.001)	−0.14 (0.08)
Serum calcium (mg/dl)		−0.17 (0.004)	−0.22 (<0.001)
PTH (pg/ml)			0.78 (<0.001)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone.

Table 4A. Multiple regression models for flow-mediated dilatation (in %) in CKD male patients: Total testosterone

	Unadjusted (β, P) ($r^2 = 0.32$)	Model 1 (β, P) ($r^2 = 0.33$)	Model 2 (β, P) ($r^2 = 0.72$)	Model 3 (β, P) ($r^2 = 0.77$)
Total testosterone (nmol/L)	0.57 (<0.001)	0.55 (<0.001)	0.26 (<0.001)	0.25 (<0.001)
Age (years)		−0.01 (0.88)	0.02 (0.49)	0.02 (0.62)
BMI (kg/m^2)		0.10 (0.06)	0.01 (0.60)	0.01 (0.70)
Total cholesterol (nmol/L)		−0.01 (0.08)	−0.05 (0.12)	−0.03 (0.31)
Diabetes (presence)		−0.06 (0.23)	−0.10 (0.007)	−0.10 (0.006)
Previous CVD (presence)		0.01 (0.90)	−0.02 (0.64)	−0.01 (0.57)
Systolic BP (mmHg)		−0.03 (0.59)	−0.10 (0.005)	−0.08 (0.02)
Diastolic BP (mmHg)		−0.02 (0.70)	0.03 (0.33)	0.02 (0.61)
Smoking (yes)		−0.09 (0.13)	−0.07 (0.04)	−0.06 (0.07)
eGFR (ml/min per 1.73 m^2)			0.68 (<0.001)	0.47 (<0.001)
Proteinuria (g/day)			−0.02 (0.48)	0.01 (0.79)
CRP (mg/L)				0.09 (0.08)
Albumin (g/dl)				0.10 (0.008)
Serum calcium (mg/dl)				−0.02 (0.56)
Serum phosphate (mg/dl)				−0.01 (0.77)
PTH (pg/ml)				−0.31 (<0.001)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; BMI, body mass index; CRP, C-reactive protein; PTH, parathyroid hormone.

Table 4B. Multiple regression models for flow-mediated dilatation (in %) in CKD male patients: Free testosterone

	Unadjusted (β, P) ($r^2 = 0.20$)	Model 1 (β, P) ($r^2 = 0.21$)	Model 2 (β, P) ($r^2 = 0.69$)	Model 3 (β, P) ($r^2 = 0.74$)
Free testosterone (10 pg/ml)	0.45 (<0.001)	0.44 (<0.001)	0.19 (<0.001)	0.16 (<0.001)
Age (years)		0.02 (0.61)	0.03 (0.34)	0.02 (0.41)
BMI (kg/m^2)		0.09 (0.10)	0.01 (0.77)	0.01 (0.88)
Total cholesterol (nmol/L)		0.02 (0.75)	−0.04 (0.19)	−0.03 (0.39)
Diabetes (presence)		−0.06 (0.26)	−0.10 (0.01)	−0.10 (<0.001)
Previous CVD (presence)		−0.01 (0.09)	−0.06 (0.08)	−0.06 (0.09)
Systolic BP (mmHg)		−0.03 (0.57)	−0.11 (0.004)	−0.10 (0.01)
Diastolic BP (mmHg)		−0.06 (0.32)	0.02 (0.55)	0.01 (0.76)
Smoking (yes/no)		−0.07 (0.24)	−0.06 (0.09)	−0.05 (0.13)
eGFR (ml/min per 1.73 m^2)			0.72 (<0.001)	0.48 (<0.001)
Proteinuria (g/day)			−0.02 (0.53)	0.01 (0.80)
CRP (mg/L)				0.05 (0.30)
Albumin (g/dl)				0.08 (0.03)
Serum calcium (mg/dl)				0.03 (0.51)
Serum phosphate (mg/dl)				0.02 (0.75)
PTH (pg/ml)				−0.33 (<0.001)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; BMI, body mass index; CRP, C-reactive protein; PTH, parathyroid hormone.

tolic and diastolic BP, and smoking) did not produce any change in the correlation coefficient of the association. Further adjustment for indicators of renal function (model 2: eGFR and proteinuria) produced an important decrease in the strength of the FMD-testosterone correlation, but this remained nonetheless significant even after further adjustment for phenotypical characteristics of CKD patients that relate to FMD (model 3: CRP, albumin, calcium, phosphate, and PTH). The same independent association was obtained for free testosterone (Table 4B). As a sensitivity analysis, and because inclusion of diabetic patients in this analysis may confound the association between testosterone and FMD measurements, we repeated the linear regression models after excluding 53 diabetic men (n = 186). Results did not change, and total and free testosterone concentration still significantly contributed to explain the variance of FMD in fully adjusted models (not shown).

Testosterone and Cardiovascular Events

Cardiovascular events were determined from the day of examination onward, with a mean follow-up period of 31 months (range 8 to 35 months). Twenty-four patients died: 22 from cardiovascular causes, 1 from infectious complications, and 1 from malignancies. Causes of cardiovascular death were coronary heart disease (n = 14), sudden death (n = 3), stroke (n = 4), or complicated peripheral vascular disease (n = 1). During the follow-up period, 50 additional nonfatal cardiovascular events were registered as follows: stroke (n = 13), myocardial infarction (n = 29), peripheral vascular disease (n = 6), and aortic aneurysm (n = 2).

The effect of total and free testosterone in the prediction of future cardiovascular events ($n = 72$, including a composite of fatal and nonfatal events) was studied by univariate and multivariate Cox analysis with gradual adjustment (Table 5). In crude analysis, every nanomole per liter of increase in total testosterone concentration reduced the risk of suffering a cardiovascular event during follow-up by 22% (crude HR 0.78 [95% CI 0.74 to 0.83]). This reduced risk persisted (HR 0.81 [95% CI 0.75 to 0.85]) after adjustment for age (per year), eGFR (per ml/min), diabetes mellitus, previous history of CVD, CRP (per mg/L), and serum albumin (per g/dl) and was not modified by adjustment for FMD values (HR 0.83 [95% CI 0.78 to 0.88]). Similar results were obtained for free testosterone values.

Discussion

To our knowledge, this is the largest screening of testosterone levels in male nondialysis CKD patients. Whereas the occurrence of testosterone deficiency is estimated to vary from 6% to 9.5% in community-dwelling men aged 40 to 75 years, rising to 15% to 30% in diabetic or obese men (15,16), our study shows a much higher prevalence in CKD patients. The overall prevalence of hypogonadism in our cohort was 33%. However, when CKD stages were looked at separately, hypogonadism increased from 17% in CKD stage 1 to 57% of the patients in CKD stage 5. The latter prevalence in CKD stage 5 patients is in agreement with previous reports on hypogonadism in Turkish (8), Swedish (7,17), and Greek (9) men undergoing dialysis. A surprising finding in our study is that the free testosterone fraction was more reduced than anticipated, observing a higher prevalence of hypogonadism if we base the definition on the free fraction. Because this is the first study, to our knowledge, to measure the free fraction in CKD patients by commercial kits and not by mass action equations, our data warrant confirmation. Because the binding capacity and concentration of sex hormone-binding globulin is traditionally thought to be normal (18), this may indicate impairment of gonadal steroidogenesis. The pathophysiological mechanisms causing the high prevalence of male hypogonadism in CKD are nevertheless not fully understood. Dysfunction of the hypothalamic-pituitary-testicular axis exists, and decreased synthesis and secretion of testosterone follow with progressive CKD (3,4,18). Comorbid conditions commonly encountered in CKD patients (*e.g.*, diabetes mellitus and hypertension) may also contribute to low testosterone levels. In fact, patients with hypogonadism in our study were more often diabetic and had a previous history of CVD. At the same time, and according to our medical records, no patients were taking androgen medication, which illustrates how this condition may be overlooked and undertreated in CKD.

Table 5A. Crude and adjusted hazard ratios of total and free testosterone concentration for prediction of cardiovascular events ($n = 72$) in CKD male patients: Total testosterone

Model		HR (95% CI)
1	Crude risk of total testosterone (per nmol/L)	0.78 (0.74 to 0.83)
2	1 + age, eGFR, diabetes, previous CVD, CRP, serum albumin	0.81 (0.75 to 0.85)
3	2 + flow-mediated dilation	0.83 (0.78 to 0.88)

HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein; CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated GFR.

Table 5B. Crude and adjusted hazard ratios of total and free testosterone concentration for prediction of cardiovascular events ($n = 72$) in CKD male patients: Free testosterone

Model		HR (95% CI)
1	Crude risk of free testosterone (per 10 pg/ml)	0.53 (0.44 to 0.62)
2	1 + age, eGFR, diabetes, previous CVD, CRP, serum albumin	0.57 (0.47 to 0.70)
3	2 + flow-mediated dilation	0.65 (0.53 to 0.80)

HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein; CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated GFR.

A recent study from Meng *et al.* (19) reported an unexpected inverse association between testosterone levels and serum phosphorus in two large, independent community-based cohorts of older men, even after adjustment for eGFR and estradiol. The authors speculated that this association may reflect enhanced deposition of phosphorus in the bone, reduced bone resorption, or previously unrecognized effects of testosterone on gastrointestinal absorption or renal excretion of phosphorus. Our analysis in etiologically diagnosed nondialysis CKD patients cannot confirm this finding because the association between testosterone and phosphate was largely dependent on PTH. It is possible that bone mineral disorders in uremia may override the associations of endogenous testosterone and phosphate observed at a community level. However, testosterone levels have been negatively associated with bone mineral disorders and receptor activator of nuclear factor κ-B ligand levels in men undergoing hemodialysis (20).

In CKD, as well as in CVD, there is a clear anabolic-catabolic imbalance, with testosterone deficiency likely further contributing to the procatabolic uremic milieu in men (5,21). It is plausible that this hormonal derangement may represent a poor prognostic sign and contribute to significant symptoms, as proposed for chronic heart failure (22). In fact, emerging evidence relates testosterone deficiency with atherosclerotic complications and CVD in various nonrenal pathologies (5,6,23,24). A proposed mechanism for the involvement of testosterone deficiency in the atherogenic process involves impaired vasodilation through decreased nitric oxide release or direct calcium antagonism in vascular endothelial and smooth muscle cells (5,25). In agreement with this experimental evidence, our study shows a strong, direct, and independent association between endogenous

testosterone (total and free forms) and endothelial function as assessed by FMD. Exclusion of diabetic patients (a potential confounder in association analysis with FMD) did not affect the independency or strength of these associations. Karakitsos *et al.* also observed an association between testosterone and FMD in 50 nondiabetic men undergoing hemodialysis (9).

Our study also demonstrates an association between endogenous testosterone concentration and the prediction of future cardiovascular events. Specifically, the risk of having a cardiovascular event during follow-up decreased by 17% for each nanomole-per-liter increment of total testosterone, irrespective of several potential confounders. Similar results were also found for free testosterone forms. To our knowledge, this is the first study looking at testosterone concentration *versus* cardiovascular outcomes in nondialysis CKD, and it complements previous reports linking testosterone with mortality in dialysis patients (7,8,17). However, contrary to our hypothesis, adjustment for FMD values did not significantly affect this relationship, implying that the association between testosterone and cardiovascular outcomes is not fully explained by mechanisms related to impaired vasodilation. Other potential pathophysiological mechanisms to explain this effect and that cannot be tested in this patient material may be related to a hypogonadism-induced procatabolic environment, including reduced erythropoiesis, low muscle mass and muscle strength, frailty, fatigue, and depression (26–28).

The study presented here has several strengths and limitations that merit consideration. Strengths of this study are the relatively large sample size of uniformly distributed etiologically diagnosed CKD patients across the different disease stages, together with the exclusion of drugs that may confound the interpretation of the eGFR-vascular health axis. Because some of these medications (*e.g.*, ACEIs/ARBs or statins) have also been suggested to affect gonadal function (29,30), this represents another strength in the assessment of our associations between testosterone and FMD. However, because of these medical exclusions, our data are not necessarily representative of the normal population of CKD patients, which should be taken as a limitation. Because eGFR is subjected to inaccuracies in CKD classification, we included only etiologically diagnosed patients, and eGFR was treated as a continuous variable in all of our analyses. Although the use of continuous variables reduces residual confounding in our analysis, we cannot exclude the possibility of other unknown confounders. Hypogonadism was diagnosed on the basis of serum testosterone levels, being unable to add clinical symptoms. Nevertheless, because many of the signs and symptoms of hypogonadism are insidious in onset and can accompany normal aging and CKD, current guidelines advocate diagnosis of hypogonadism in CKD patients primarily on the basis of laboratory testing (31). Testosterone was assessed after an overnight fasting, thereby reducing circadian variability (31). However, the measurements are based on RIA methods, which have its limitations against the gold standard of liquid chromatography-mass spectrometry. Finally, because cardiovascular events were retrieved from medical records, we cannot exclude the possibility of unreported events. Altogether, this may result in underestimation of the observed effects. Ultimately, we want to emphasize that our study design does not allow to causally linking these associations; testosterone may also be a marker of chronic disease.

To conclude, our observational cohort study provides a comprehensive overview of the evolution of testosterone levels in men with progressive kidney failure. We show that total and free testosterone concentration fall in parallel with the decline of eGFR, appearing as important and independent determinants of FMD in multivariate analyses. Finally, total and free testosterone emerged as predictors of future cardiovascular outcomes in nondialysis CKD patients. This and previous observational evidence may trigger further research to confirm or reject the hypothesis of a causative relationship between this gonadotropin hormone and cardiovascular outcomes in male CKD patients and incite interventional studies to treat this modifiable endocrine disorder. All in all, this study emphasizes that different hormonal and metabolic disturbances may take part in men and women with CKD, ultimately underlining the need for individualized and sex-specific therapeutic care and management (32).

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Disclosures

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