Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20–79

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Aims	Although the association of low serum testosterone levels with mortality has gained strength in recent research, there are few population-based studies on this issue. This study examined whether low serum testosterone levels are a risk factor for all-cause or cause-specific mortality in a population-based sample of men aged 20–79.
Methods and results	We used data from 1954 men recruited for the prospective population-based Study of Health in Pomerania, with measured serum testosterone levels at baseline and 195 deaths during an average 7.2-year follow-up. A total serum testosterone level of less than 8.7 nmol/L (250 ng/dL) was classified as low. The relationships of low serum testosterone levels with all-cause and cause-specific mortality were analysed by Cox proportional hazards regression models. Men with low serum testosterone levels had a significantly higher mortality from all causes than men with higher serum testosterone levels (HR 2.24; 95% CI 1.41–3.57). After adjusting for waist circumference, smoking habits, high-risk alcohol use, physical activity, renal insufficiency, and levels of dehydroepiandrosterone sulfate, low serum testosterone levels continued to be associated with increased mortality (HR 2.32; 95% CI 1.38–3.89). In cause-specific analyses, low serum testosterone levels predicted increased risk of death from cardiovascular disease (CVD) (HR 2.56; 95% CI 1.15–6.52) and cancer (HR 3.46; 95% CI 1.68–6.68), but not from respiratory diseases or other causes.
Conclusion	Low serum testosterone levels were associated with an increased risk of all-cause mortality independent of numerous risk factors. As serum testosterone levels are inversely related to mortality due to CVD and cancer, it may be used as a predictive marker.
Keywords	Testosterone • All-cause and CVD mortality • Men • Study of Health in Pomerania (SHIP)

Introduction

Serum testosterone levels decline after the starting age of 30 about 1–2% per year.[1] Previous research suggests that low serum testosterone levels are associated with multiple risk factors for cardiovascular disease (CVD), including hypertension, abdominal obesity, insulin resistance, thrombosis, and inflammatory markers.[2] Further, low serum testosterone levels are associated with decreased bone mineral density and osteoporosis,[3] decreased libido and energy,[4] or chronic fatigue.[5] Thus, low serum testosterone levels are associated with medical conditions that are themselves associated with significant morbidity and increased mortality. The association of low serum testosterone levels and mortality has been investigated in few population-based studies, which demonstrated conflicting results. Recent reports from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk),[6] the Rancho Bernado,[7] and

the osteoporotic fractures in men (MrOS) Sweden study[8] demonstrated that older men with lower serum testosterone levels had a greater risk of dying, compared with men with higher serum testosterone levels. In contrast, two other prospective cohort studies reported no association between low serum testosterone levels and premature death.[9,10] These studies raise important issues related to measuring testosterone for aetiological and clinical use.[11] Our study has three aims. First, we seek to characterize the group of men with low serum testosterone levels. Second, we want to investigate if there is an association of low serum testosterone levels with all-cause or cause-specific mortality in a prospective population-based cohort of 1954 men aged 20–79. Third, we aim to identify a cut-off for the definition of low serum testosterone by exploring the highest predictive value in terms of mortality risk.

Subjects and methods

Study population

Data from the Study of Health in Pomerania (SHIP) were used.[12,13] The target population were adult German residents of West Pomerania in north-eastern Germany. The study conformed to the principles of the Declaration of Helsinki as reflected by an *a priori* approval of the Ethics Committee of the University of Greifswald. From 2117 male baseline participants (response 69%), we excluded 70 men who used opiates [anatomic–therapeutical–chemical (ATC) code N02AA, A07DA02, R05DA, R05FA0; n = 11] or glucocorticoids (ATC code R03BA; n = 31, H02AB; n = 28) from analysis. Furthermore, men who received sexual hormones (ATC code G03; n = 2), testosterone 5α-reductase inhibitors (ATC code G04CB; n = 4), or sexual hormone antagonists (ATC code L02B; n = 1) were not considered. None of the subjects reported taking anabolic steroids (ATC code A14A). Among the remaining 2040 men, one subject with serum testosterone >55.5 nmol/L (>1599.5 ng/dL), nine subjects with serum testosterone <0.69 nmol/L (<19.9 ng/dL), and a further of 76 subjects with no blood drawn or no values for serum testosterone were also excluded. This resulted in a final study population of 1954 men.

Measures

Socio-demographic and behavioural characteristics and medical history were assessed by computer-aided personal interviews. We considered age, educational level (<10, 10, or >10 years of schooling), and cohabitation (cohabiting or living alone). Mean daily alcohol consumption was calculated using beverage-specific pure ethanol volume proportions.[14] Riskful alcohol consumption was classified as >30 g alcohol/day. Smoking habits were assessed by dividing men into categories of current, former, and never smokers. Men who participated in physical training during summer or winter for at least 1 h a week were classified as being physically active.

Waist circumference (WC) was measured to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane, with the subject standing comfortably with weight distributed evenly on both feet. Height was measured to the nearest 1 cm using a digital ultrasound instrument and weight was measured to the nearest 0.1 kg in light clothing and without shoes using standard digital scales

(Soehnle-Waagen GmbH, Nassau, Germany). Body mass index was calculated as weight in kilograms divided by the square of height in metres. Hypertension was defined as elevated systolic (≥140 mmHg) or diastolic (≥90 mmHg) blood pressure or the use of hypertensive medication (ATC code C02). Diabetes mellitus, chronic bronchitis, myocardial infarction, and stroke were identified from self-reported physician's diagnoses.

Assays

A blood sample was drawn from the cubital vein in the supine position. The samples were taken between 7 a.m. and 4 p.m. and serum aliquots were prepared for immediate analysis and for storage at −80°C for further analysis. From fresh serum, creatinine levels were determined with the Jaffé method (Hitachi 717, Roche Diagnostics, Germany). Creatinine clearance (CrCl) was estimated using the Cockroft–Gault formula.[15] Renal insufficiency was defined on self-reported renal diseases or CrCl < 50 mL/min. Serum low-density lipoprotein cholesterol was measured applying a precipitation procedure using dextran sulphate (Immuno, Heidelberg, Germany) on an Epos 5060 (Eppendorf, Hamburg, Germany). Hyperlipidaemia was defined by a serum low-density lipoprotein cholesterol level >3.88 mmol/L or lipid medication (ATC code C10ab). Dehydroepiandrosterone sulfate (DHEAS) and total testosterone levels were measured from frozen serum aliquots using competitive chemiluminescent enzyme immunoassays on an Immulite 2500 analyzer (Siemens Immulite 2500 DHEA-SO4, ref. L5KDS, lot 106; Siemens Immulite 2500 Total Testosterone, ref. L5KTW, lot 110; Siemens Healthcare Medical Diagnostics, Bad Nauheim, Germany). Measurements of testosterone were performed during December 2005 and January 2006. An aliquot of two alternating levels of a third-party commercial control material (Bio-Rad Lyphochek Immunoassay Plus Control, lot 40151 and lot 40152; Bio-Rad, Munich, Germany) was included in each series in single determination. The inter-assay coefficient of variation was 13.2% with a systematic deviation of +2.3% at the 3.2 nmol/L level and 8.9% with a systematic deviation of +0.24% at the 22.5 nmol/L level. Serum testosterone levels below 8.7 nmol/L (250 ng/dL) were considered as low. All assays were performed according to the manufacturers' recommendations by skilled technical personal. In addition, the laboratory takes part in official quarterly German external proficiency testing programs.

Follow-up of vital status

Information on vital status was acquired at regular intervals from the time of enrolment into the study through 31 August 2007. Subjects were included in the analysis until the time of either death or when they were censored due to study end or failure to follow-up. The number of months between baseline examination and censoring was used as follow-up length. The mean duration of follow-up was 7.2 years (25th, 6.6; 75th: 8.0). Death certificates were requested from the local health authority of the residence of death and were coded by a certified nosologist according to the International Classification of Diseases, 10th revision (ICD-10). Additionally, two internists (H.W. and M.D.) independently validated the underlying cause of death, and performed a joint reading in cases of disagreement. A third internist (H.V.) finally decided in cases of still existing disagreement.

Statistical analysis

Categorical data are expressed as percentages and continuous data are expressed as medians (25th; 75th percentiles). Univariate analysis was performed with χ^2 testing for categorical variables, and a *t*-test (Mann−Whitney *U* test for non-normal distributions) for continuous variables. To assess the association between serum testosterone levels and mortality, we used Cox proportional hazards regression models. Different stepwise models were implemented adjusted for age, WC, smoking habits, high-risk alcohol use, and physical activity, as well as renal insufficiency and DHEAS levels. All models were rerun for cause-specific mortality analyses. *P*-value for trend was calculated with continuous serum testosterone levels.

The following sensitivity analyses were conducted. First, interaction was assessed by a product term of continuous serum testosterone levels with covariates and was kept in the models for age, WC, and DHEAS ($P < 0.05$). Second, to adjust for possible bias introduced by informative censoring, inverse probability weighting was used.[16] Third, we excluded first-year deaths. And finally, the sample was stratified by blood sampling time. Models testing for mediation estimated the association of serum testosterone with mortality in multivariable Cox models, controlling for the intermediate variable each in a separate model. We used both graphical and hypotheses testing methods for examining the proportional hazard assumption.[17] Schoenfeld's tests as well as visual inspection of smoothed estimates of the hazard ratio against time confirmed the proportional hazard assumption for all variables. Kaplan−Meier survival curves were used to illustrate the association of serum testosterone level and all-cause mortality, with differences tested by a log-rank test. To compare the predictability of testosterone along with standard mortality risk factors (age, smoking habits, alcohol consumption, physical activity, and WC), we estimated area under receiver operating characteristic curves (AUCs area) from fully adjusted Cox models and tested their difference. *A posteriori* power analyses were calculated using an nQuery Advisor program (nQuery Advisor 6.0, Statistical Solutions, Cork, Ireland). Hazard ratios were calculated with a 95% confidence interval. A value of $P < 0.05$ was considered statistically significant. All statistical analyses were performed using Stata 9 (Stata Corporation, College Station, TX, USA). This manuscript was written in accordance with the STROBE statement, giving guidelines for reporting of observational studies.[18]

Results

During the 13 913 person-years of follow-up, 195 men had died, reflecting a death rate of 14 deaths per 1000 person-years. Men with low serum testosterone levels were significantly older, exposed higher body fat accumulation, smoked less, appeared to be physically less active, and showed higher prevalences of diabetes and metabolic syndrome than men with higher serum testosterone levels (*Table 1*). Furthermore, men with low serum testosterone levels had significantly lower serum DHEAS levels and higher serum creatinine levels compared with men with higher serum testosterone levels. There were no significant differences concerning cohabitation, educational level, LDL-cholesterol, high-risk alcohol

use, or prevalences of chronic bronchitis, myocardial infarction, or stroke. Decedents exposed significantly lower serum testosterone levels compared with survivors (*Figure 1*) and differed also in all of the other considered characteristics except for prevalent hypertension (*Table 1*).

Cox proportional hazard models revealed an association of low serum testosterone levels with increased risk of mortality (*Table 2*). This association was independent of age (HR 2.24; 95% CI 1.41−3.57), WC (HR 2.10; 95% CI 1.34−3.29), smoking habits, high-risk alcohol use, and physical activity (HR 2.32; 95% CI 1.38−3.89), as well as renal insufficiency and DHEAS levels (HR 1.92; 95% CI 1.18−3.14). Continuous levels of serum testosterone were not associated with increased mortality (*P* for trend >0.05). The Kaplan−Meier analysis in *Figure 2* confirmed the revealed association, showing that men with low serum testosterone levels had shorter survival times than men with higher serum testosterone levels (log rank test; $P < 0.001$). Age-specific analyses revealed an increased risk of premature death in all age groups, but with significant estimates only in the elderly (60−79 years) (*Table 2*). Mediating effects were detected only for renal insufficiency attenuating the estimates slightly (*Table 3*). Estimates did not change substantially after excluding first-year deaths (HR 2.86; 95% CI 1.67−4.89). In cause-specific mortality analysis, low serum testosterone levels were significantly associated with an increased mortality caused by CVD (HR 2.56; 95% CI 1.15−6.52) and cancer (HR 3.46; 95% CI 1.68−6.68), but not for respiratory diseases or other causes (*Table 2*).

Sensitivity analysis with sample stratified by blood sampling time confirmed the revealed association of low serum testosterone levels with mortality in men with blood drawn before 11 a.m. (*Table 2*). Further sensitivity analysis of the testosterone−mortality association by different cut-offs from recent studies revealed that serum testosterone levels between 8.0 nmol/L (230 ng/dL) and 8.7 nmol/L (250 ng/dL) were consistently associated with increased mortality, whereas levels below 6.94 nmol/L (200 ng/dL) or above 8.7 nmol/L (250 ng/dL) showed inconsistent results (*Table 4*). To estimate the possible bias introduced by informative censoring, we re-analysed the re-weighted study sample. But the revealed estimates decreased only little compared with the fully adjusted models, suggesting a minor confounding effect of informative censoring (*Table 2*). Analysing predictability, we found that testosterone only slightly improves the risk prediction of mortality (AUCs area of 0.79) compared with standard mortality risk factors (AUCs area of 0.78), although the tested differences were significant ($P = 0.047$). Implementing significant interaction terms for testosterone with age, WC, and DHEAS to the conducted modelling, we revealed slightly increased estimates (*Table 2*). Alternatively, assessing dichotomized interaction terms of serum testosterone levels with bivariate covariates, we were not able to identify any significant dichotomous interaction coefficients. But when we additionally assessed the potential interaction of testosterone with age by analysis of a 2×2 table,[19] we revealed that old men (≥60 years) with low serum testosterone levels exposed the highest mortality risk (*Figure 3*). Even more interestingly, men with low serum testosterone levels showed higher risk of mortality compared to men with higher testosterone levels in both age groups (*Figure 3*). We performed a posteriori power analyses to

Table 1 Baseline characteristics of men stratified by serum testosterone levels

	Testosterone level; <8.7 nmol/L (n = 98)	Testosterone level; ≥8.7 nmol/L (n = 1856)	Decedents (n = 193)	Survivors (n = 1761)
Age (years)	63.1 (52.3; 79.2)*	58.5 (44.1; 71.4)	75.0 (68.2; 79.5)*	56.8 (43.5; 69.7)
Cohabiting	85.6	80.1	78.7*	80.6
Low educational level (<10 years)	47.4	41.8	75.4*	38.5
Waist circumference (cm)	100.6 (94.1; 110.5)*	95.0 (87.2; 102.5)	100.1 (92.4; 107.5)*	94.8 (87.0; 102.5)
Body mass index (kg/m^2)	28.8 (27.3; 31.9)*	27.3 (24.8; 29.9)	28.2 (25.7; 30.8)*	27.3 (24.9; 29.9)
Serum total testosterone (nmol/L)	7.6 (5.9; 8.1)*	16.3 (13.2; 20.4)	14.2 (11.5; 20.2)*	17.0 (12.8; 20.1)
Serum DHEAS (μg/mL)	1.2 (0.8; 1.7)*	1.7 (1.0; 2.6)	1.0 (0.6; 1.5)*	1.7 (1.1; 2.7)
Creatinine >150 μmol/L	5.2*	0.7	5.8*	0.4
LDL cholesterol (mmol/L)	3.6 (2.7; 4.4)	3.6 (2.8; 4.3)	3.7 (3.0; 4.4)*	3.5 (4.3; 2.8)
High-risk alcohol use (%)	21.6	23.6	14.6*	24.4
Smoking				
Never smoker	23.7*	20.9	21.8*	14.6
Ex-smoker	56.7*	43.9	43.6*	53.5
Current smoker	19.6*	35.1	34.6*	31.9
Hypertension	43.3*	27.9	30.1	28.5
Physical activity	31.9*	42.5	27.6*	43.5
Chronic bronchitis	2.1	5.0	10.3*	4.3
Diabetes mellitus	20.4*	5.6	16.1*	5.3
Metabolic syndrome	61.5*	33.4	51.1*	33.3
Myocardial infarction	6.3	5.5	12.0*	4.9
Stroke	2.1	3.1	12.5*	2.1

Data are percentages or median (25th; 75th).
DHEAS, dehydroepiandrosterone sulfate; LDL, low-density lipoprotein. To convert the values of serum testosterone to ng/dL multiply by 28.82.
*$P < 0.05$ for the overall difference in means based on the χ^2 test or the analysis of variance (U test or t-test) for men with testosterone levels <8.7 vs. ≥8.7 nmol/L and decedents vs. survivors.
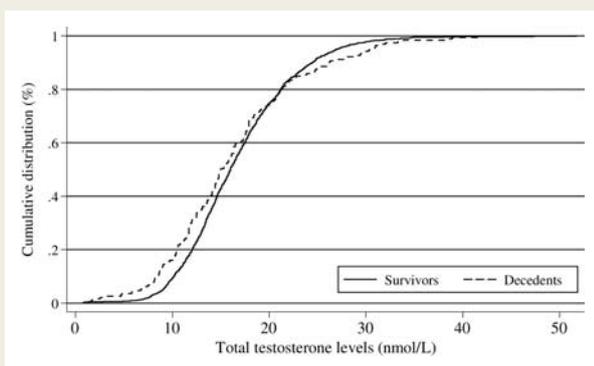
Figure 1 Cumulative distribution function of continuous serum testosterone levels by vital status.

assess the minimum detectable relative risk for mortality in the comparison between men with low and higher serum testosterone levels. Given the real proportion of men with low testosterone levels, our study size was large enough to detect a minimum relative risk for mortality of 2.22 with a power of 80% (two-sided χ^2 test; $\alpha = 5\%$), whereas the present analyses detected a real relative risk of 3.28 with a power of 97%.

Discussion

To the best of our knowledge, this is the first prospective population-based study that confirmed the previously reported association between low serum testosterone levels and mortality.[6–8] We showed that in a sample of men aged 20–79 men with serum testosterone levels below 8.7 nmol/L (250 ng/dL) had a more than two-fold increased risk of mortality from all causes compared with those with higher serum testosterone levels, independent of age, WC, smoking habits, high-risk alcohol use, and physical activity. In contrast, low serum testosterone levels were not associated with premature death in two other prospective cohort studies, the Massachusetts Male Aging study (MMAS),[9] the Caerphilly study,[10] and InCHIANTI reporting that low concentrations of a combination of hormones, but not testosterone alone, were associated with death.[20] Possible explanations, why three of six recent studies failed to find an association of serum testosterone with mortality are differences in follow-up times, age structure, cut-off for the definition of low serum testosterone levels, as well as assay methods.

The mean follow-up time in studies reporting a negative association (16.5,[10] 15.3,[9] and 6 years,[20] respectively) was comparable with studies reporting a positive association (7.0,[6] 11.8,[7] and 4.5 years[8]) and about two times longer than ours (7.2 years). Therefore, distinct

Table 2 Multivariable models for low serum testosterone levels associated with all-cause and cause-specific mortality

Cause of death (ICD-10 codes)	Number of deaths	Person-years	Crude incidence rate (per 1000 person-years)	HR (95% CI)			
				Model 1	Model 2	Model 3	Model 4
All-cause (A00—T98) (n = 1954)	193	13 913.5	14.0	2.24 (1.41; 3.57)**	2.10 (1.34; 3.29)**	2.32 (1.38; 3.89)**	1.92 (1.18; 3.14)**
P trend				0.274	0.118	0.292	0.171
20−59 years (n = 1018)	24	7455.8	3.2	2.86 (0.84; 9.71)	3.05 (0.87; 10.78)	3.04 (0.97; 11.98)	2.15 (0.58; 7.96)
60−79 years (n = 936)	169	6457.7	26.2	2.08 (0.30; 3.13)**	2.03 (1.28; 3.26)**	2.25 (1.40; 3.60)**	2.18 (1.27; 3.74)**
CVD (I10−I79)	68	13 381.5	5.2	2.95 (1.10; 7.94)*	2.49 (1.17; 5.31)*	2.84 (1.13; 7.16)*	2.56 (1.15; 6.52)*
Respiratory disease (J00−J99)	11	13 120.1	0.8	1.14 (0.14; 9.22)	0.98 (0.12; 8.00)	1.57 (0.18; 13.63)	1.46 (0.20; 10.59)
Cancer (C00−C97)	73	13 355.5	5.5	2.99 (1.16; 7.71)*	3.13 (1.57; 6.21)**	3.64 (1.77; 7.46)***	3.46 (1.68; 6.68)**
Other causes	40	13 224.9	3.0	1.37 (0.41; 4.59)	1.33 (0.40; 4.46)	1.63 (0.48; 5.57)	1.50 (0.53; 5.16)
Excluding first-year deaths (n = 1934)							
All cause (A00−T98)	173	13 904.3	12.4	2.19 (1.39; 3.46)**	2.17 (1.23; 3.83)**	2.44 (1.54; 3.88)***	1.97 (1.19; 3.26)**
Sample stratified by blood sampling time before and after 11 o'clock							
<11 a.m. (n = 1197)	109	8539.6	12.8	2.09 (1.10; 3.99)*	2.05 (1.07; 3.92)*	2.05 (1.06; 3.96)*	2.13 (1.14; 3.97)*
≥11 a.m. (n = 757)	84	5366.1	15.7	1.57 (0.88; 2.82)	1.52 (0.85; 2.74)	1.82 (1.00; 3.29)*	1.44 (0.63; 3.29)
Interaction terms included for age, WC, and smoking							
All cause (A00−T98) (n = 1954)	193	13 913.5	14.0	2.91 (1.75; 4.85)***	3.05 (1.83; 5.09)***	3.25 (1.94; 5.45)***	3.23 (1.84; 5.67)***
Inclusion of inverse probability weights							
All cause (A00−T98) (n = 1954)	193	13 913.5	14.0	2.73 (1.61; 4.62)***	2.80 (1.66; 4.75)***	2.79 (1.64; 4.75)***	2.77 (1.54; 4.97)***

Model 1: adjusted for age. Model 2: adjusted for age, and WC. Model 3: adjusted for model 2, smoking (three categories), high-risk alcohol use, and physical activity. Model 4: adjusted for model 3, renal insufficiency, and DHEAS. HR, hazard ratio; CI, 95% confidence interval; CVD, cardiovascular disease; WC, waist circumference; DHEAS, dehydroepiandrosterone sulfate.
*P < 0.05.
**P < 0.01.
***P < 0.001.

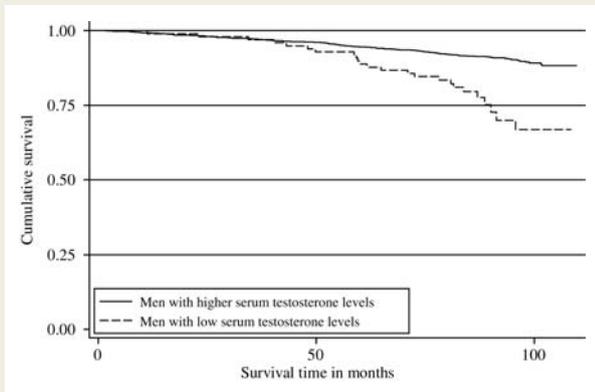**Figure 2** Kaplan–Meier survival curves for low serum testosterone levels <8.7 nmol/L (<250 ng/dL) and higher serum testosterone levels >8.7 nmol/L (>250 ng/dL). Legend: Men with low serum testosterone levels had a significantly shorter survival than men with higher serum testosterone levels (log-rank test; $P = .001$).

Table 3 Hazard ratios for low serum testosterone levels associated with all-cause mortality adjusted for potential mediators

	Testosterone level <8.7 nmol/L (250 ng/dL) HR (95% CI)
Model 3[†]	2.32 (1.38; 3.89)**
+ Hypertension	2.36 (1.50; 3.71)***
+ Diabetes mellitus	2.23 (1.38; 3.59)**
+ Metabolic syndrome	2.36 (1.49; 4.06)***
+ Myocardial infarction	2.37 (1.45; 3.87)**
+ Renal insufficiency	2.06 (1.19; 3.53)**
+ Hyperlipidaemia	2.40 (1.49; 3.85)***
+ Cohabitation	2.41 (1.53; 3.79)***
+ Educational level	2.31 (1.48; 3.61)***
+ Stroke	2.41 (1.50; 3.89)***
+ DHEAS	2.77 (1.68; 4.58)***
+ Blood sampling time	2.13 (1.37; 3.90)**

Covariables were added one at a time to model 3. HR, hazard ratio; CI, 95% confidence interval; DHEAS, dehydroepiandrosterone sulfate; WC, waist circumference.
[†]Model 3: adjusted for age, WC, smoking (three categories), high-risk alcohol use, and physical activity.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

Table 4 Association of low testosterone levels with all-cause mortality by different cut-offs from recent studies

Cut-off for the definition of low total testosterone (TT)	MMAS;[8] TT <6.94 nmol/L (200 ng/dL)	Wang;[34] TT <8.0 nmol/L (230 ng/dL)	Rancho Bernardo;[7] TT <8.36 nmol/L (241 ng/dL)	Male Veterans Study;[35] TT <8.7 nmol/L (250 ng/dL)	HIM;[36] TT <10.41 nmol/L (300 ng/dL)	EPIC;[6] TT <12.5 nmol/L (360 ng/dL)	Age-specific cut-off <10th percentile
Low TT (n)	34	69	82	98	241	474	
Model 1	1.59 (0.83; 4.02)	1.96 (0.93; 3.63)	2.21 (1.26; 3.89)**	2.24 (1.41; 3.57)**	1.33 (0.93; 1.90)	1.28 (0.95; 1.72)	2.21 (1.40; 3.49)**
Model 2	2.12 (1.01; 4.46)*	2.08 (1.12; 3.86)*	2.33 (1.33; 4.12)**	2.10 (1.34; 3.29)**	1.28 (0.89; 1.84)	1.20 (0.88; 1.62)	2.26 (1.43; 3.59)**
Model 3	2.50 (1.18; 5.27)*	2.24 (1.21; 4.17)*	2.53 (1.43; 4.47)**	2.32 (1.38; 3.89)**	1.37 (0.95; 1.99)	1.28 (1.93; 1.75)	2.35 (1.47; 3.74)***
Model 4	2.68 (1.19; 6.04)*	2.13 (1.06; 4.26)*	2.56 (1.38; 4.76)**	1.92 (1.18; 3.14)**	1.11 (0.72; 1.69)	1.10 (0.78; 1.56)	2.25 (1.35; 3.75)**

Model 1: adjusted for age. Model 2: adjusted for model 1, WC. Model 3: adjusted for model 2, smoking (3 categories), high-risk alcohol use, and physical activity. Model 4: adjusted for model 3, renal insufficiency, and DHEAS. HR, hazard ratio; CI, 95% confidence interval; CVD, cardiovascular disease; WC, waist circumference; DHEAS, dehydroepiandrosterone sulfate.
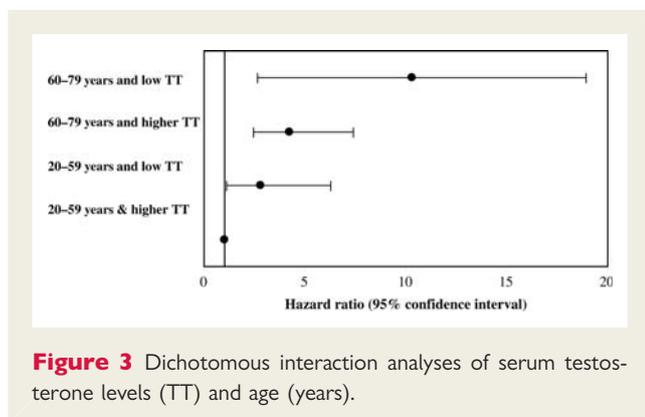*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

follow-up times cannot explain the differences between the studies. Concerning differences in age structure, populations from positive studies including ours (Rancho Bernardo (mean 74; min 50; max 91), EPIC-Norfolk (mean age 67; min 42; max 78), MrOS Sweden (mean 75; min 69; max 80), and SHIP (mean age 58; min 20; max

Figure 3 Dichotomous interaction analyses of serum testosterone levels (TT) and age (years).

79) were not older than those from negative ones [InCHIANTI (mean age 75; min 65; max 92), MMAS (mean age 55; min 40; max 70), and Caerphilly (mean age 52; min 45; max 59)]. Because the optimal testosterone concentration is still a subject of debate, arbitrary cut-offs for the definition of low serum testosterone levels could be a reason for the different findings. When we assessed the association of low serum testosterone levels with mortality by different cut-offs from recent studies, levels between 8.0 nmol/L (230 ng/dL) and 8.7 nmol/L (250 ng/dL) were most convincingly associated with increased mortality. This finding suggests that an optimal cut-point for predicting premature death may be <10.4 nmol/L (300 ng/dL). However, a long overdue agreement about the optimal serum testosterone concentration needs to be determined.[21] As long as threshold levels are arbitrary, it is nearly impossible to perform risk stratification by a certain cut-off level or to estimate the impact of low serum testosterone levels on mortality across different populations or studies. Also differences in assay methods are not likely to cause different associations: both Caerphilly[10] and MMAS[9] measured testosterone using direct radioimmunoassays. The automated immunoassay used in our study showed a higher concordance[22,23] with the latter assay and might not be the reason for differences, as previously suggested.[7] We also conducted a sensitivity analysis by stratifying our sample according to the time of blood drawn, revealing a stronger association of low serum testosterone levels with mortality in men with blood drawn before 11 o'clock. This finding is in line with the general recommendation that, due to a maintained diurnal rhythm in healthy men into the seventh decade of life,[24] serum samples for testosterone determination should be obtained between 7.00 and 11.00 a.m.[25]

Testicular function is suppressed in many acute and chronic illnesses resulting in reduced serum testosterone.[4] Low serum testosterone has been reported in type 2 diabetes,[26] chronic obstructive pulmonary disease,[27] alcoholic liver disease,[28] chronic renal disease,[29] and metabolic syndrome,[30,31] suggesting low serum testosterone levels rather a marker of pre-existing disease, than an independent risk factor of mortality. Therefore, we carefully adjusted for medical morbidity and other clinical covariables and continued to find an association between low serum testosterone levels and mortality. This finding is in line with recent results demonstrating an inverse association of serum testosterone levels with all-cause and CVD mortality in male patients with chronic kidney disease[32] or heart failure.[33] Further adjustment for serum DHEAS did not change the estimates too, although an

association of low serum DHEAS levels with increased mortality has been reported previously.[34] Sensitivity analyses without first-year deaths yielded no differences in estimates, suggesting that the association between low serum testosterone and mortality is not simply due to acute illness.

The observed association between low serum testosterone levels and mortality was not specific to a single aetiology. Low serum testosterone levels were strongly associated with deaths due to both CVD and cancer. During the past decades, reports linked differences in serum testosterone levels to various cardiovascular risk factors and also directly to CVD and death.[35] As the association of low serum testosterone levels with CVD mortality might be explained by cardiometabolic factors such as central obesity, metabolic syndrome or diabetes,[36] we performed sensitivity analysis with cardiometabolic mediator variables, but without revealing any significant impact on the estimates. The MMAS reported an association of low serum testosterone levels with mortality from cancer and respiratory disease.[9] As our results confirm the first association we could not proof the latter, maybe because we excluded 58 men from analysis who used systemic glucocorticoids which have an adverse dose-dependent effect on testosterone production.[37] Of those, 34 suffered from chronic bronchitis with 8 deaths during follow-up, making it likely that we might have underestimated respiratory disease mortality.

Limitations arise from the lack of measured free testosterone, SHBG, or albumin for calculation of bioavailable testosterone, as well as from single measurement of testosterone. But this and previous studies[6,7,9,10] were based on a single measurement of serum testosterone, which is believed to be accurate for population studies.[38] Further potential limitations may arise from short-time follow-up, as a longer follow-up length could have provided more events for cause-specific mortality analysis. Also symptoms of qualitative hypogonadism were not assessed in our study, to draw any conclusions above laboratory diagnosis of hypogonadism by low serum total testosterone levels. The major strength of our study is the use of data from a large population-based sample of men with a broad age range from 20 to 79 years, still revealing an increased risk of mortality independent of age, adiposity, and lifestyle factors. This association cannot be explained by pre-existing disease and was not specific to a single cause of death.

However, as the exact mechanism by which testosterone may cause an increased risk of death is currently not known,[35] we propose testosterone being a risk marker, rather than risk factor. A risk marker is not assumed to play an aetiological or direct causal role, but is mainly useful to improve our ability to predict risk. In that light, establishing statistically significant associations is necessary but not sufficient. Therefore, we believe that further answers about causality and pathogenesis could be inferred from long-term, double-blind, randomized, placebo-controlled trials of testosterone replacement in men with well-documented testosterone insufficiency.

Acknowledgements

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