Low Free Testosterone Predicts Mortality from Cardiovascular Disease But Not Other Causes: The Health in Men Study

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Context: Low testosterone is associated with all-cause mortality, but the relationship with cause-specific mortality is uncertain.

Objective: Our objective was to explore associations between testosterone and its related hormones and cause-specific mortality.

Design: This was a population-based cohort study.

Setting and Participants: Demographic and clinical predictors of mortality, and testosterone, SHBG, and LH were measured from 2001–2004 in 3637 community-dwelling men aged 70–88 yr (mean, 77 yr).

Main Outcome Measure: Cause of death was obtained via electronic record linkage until December 31, 2008.

Results: During a mean follow-up period of 5.1 yr, there were 605 deaths. Of these, 207 [34.2%; 95% confidence interval (CI) = 30.4–38.1%] were due to cardiovascular disease (CVD), 231 to cancer (38.2%; 95% CI = 34.3–42.1%), 130 to respiratory diseases (21.5%; 95% CI = 18.2–24.8%), and 76 to other causes (12.6%; 95% CI = 9.9–15.2%). There were 39 deaths attributable to both cancer and respiratory diseases. Lower free testosterone (hazard ratio = 1.62; 95% CI = 1.20–2.19, for 100 *vs.* 280 pmol/liter), and higher SHBG and LH levels were associated with all-cause mortality. In cause-specific analyses, lower free testosterone (sub-hazard ratio = 1.71; 95% CI = 1.12–2.62, for 100 *vs.* 280 pmol/liter) and higher LH predicted CVD mortality, while higher SHBG predicted non-CVD mortality. Higher total testosterone and free testosterone levels (sub-hazard ratio = 1.96; 95% CI = 1.14–3.36, for 400 *vs.* 280 pmol/liter) were associated with mortality from lung cancer.

Conclusions: Low testosterone predicts mortality from CVD but is not associated with death from other causes. Prevention of androgen deficiency might improve cardiovascular outcomes but is unlikely to affect longevity otherwise. **(*J Clin Endocrinol Metab* 97: 179–189, 2012)**

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Abbreviations: AR, Androgen receptor; CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; LDL, low-density lipoprotein; SHR, sub-hazard ratio; W1, wave 1; WADLS, Western Australian Data Linkage System.

Since time immemorial, the ability to resist the effects of aging and prolong life has been a universal human desire. For millennia, alchemists sought in vain for an elixir of life, while more recently, 19th-century physicians attempted to produce a rejuvenating tonic from animal testicular extracts (1).

The discovery and synthesis of sex steroids in the first half of the 20th century renewed interest in this idea, and testosterone therapy was widely promoted for the male climacteric (2, 3). However, this term is misleading, because there are clear sex differences in age-related hormonal decline. In women, a marked and abrupt reduction in estrogen and progesterone is observed during menopause, while in men, testosterone peaks in early adulthood, then decreases by a modest 1–2% per year for the remainder of life (4). Nonetheless, these changes are temporally associated with many features of aging, and debate remains as to whether decreased sex hormone output contributes to the aging process (5).

Hypogonadism is associated with components of the frailty syndrome, such as sarcopenia and decreased bone mineral density (6), but evidence is strongest for a role in the cardiovascular system. Low testosterone is associated with risk factors for cardiovascular disease (CVD), including insulin resistance, metabolic syndrome, and type 2 diabetes (7), and predicts the development of atherosclerosis and cardiovascular events (7, 8). Conversely, testosterone replacement generally improves abdominal obesity, insulin sensitivity, and the lipid profile, although decreases in high-density lipoprotein are also reported (9). Testosterone therapy has also been shown to improve physical function in elderly men with features of frailty (10).

Nonetheless, androgenic anabolic steroid abuse is associated with cardiomyopathy and sudden death (3), and a recent testosterone trial was terminated after excess cardiovascular events in the treatment group (11). Epidemiological studies also conflict. Some report low testosterone predicts all-cause mortality (12–17), while others report no association (18, 19) or that higher levels are associated with increased cardiovascular but reduced respiratory mortality (20). The ability to draw conclusions from the data is further limited by differences in age range, definition of endpoints, and in the few studies examining cause-specific mortality, failure to control for the problem of competing risks. Additionally, as far as we are aware, no previous study has examined the relationship between mortality and gonadotropins, which together with testosterone, may provide better risk stratification than testosterone levels alone (21).

We designed the present analysis to explore associations between sex hormones and cause-specific mortality in a cohort of men aged 70–88 yr at baseline. We initially examined all-cause mortality and then performed a series of subanalyses for deaths due to CVD, respiratory diseases, cancer, and all remaining causes (as a composite). We hypothesized that men with low testosterone or elevated LH would be at higher risk of all-cause mortality and that this would be attributable to CVD, but not other causes, during a follow-up period of approximately 5 yr.

Subjects and Methods

Study population

The Health in Men Study is a population-based cohort study of men aged 65 yr and older from Perth, Western Australia (22). A total of 12,203 men participated in wave 1 (W1) from 1996–1999. During wave 2 (W2) in 2001–2004, 4249 participated and provided blood samples. The University of Western Australia Human Research Ethics Committee approved the study, and all men gave written informed consent to participate.

Biochemical assessment

Blood samples were collected between 0800 and 1030 h. Biochemical assays were performed in the Biochemistry Departments of Royal Perth and Fremantle hospitals. Serum total testosterone, SHBG, and LH were determined by chemiluminescent immunoassays on an Immulite 2000 analyzer (Diagnostic Products Corp. Biomediq, Doncaster, Australia). Between-day imprecision for total testosterone was 11.2% at 7.2 nmol/liter and 8.9% at 18 nmol/liter; for SHBG it was 6.7% at 5.2 nmol/liter and 6.2% at 81 nmol/liter; and for LH it was 6.4% at 2.3 IU/liter and 5.8% at 19 IU/liter. Working ranges for these assays are 0.7–55 nmol/liter for testosterone, 2–180 nmol/liter for SHBG, and 0.1–200 IU/liter for LH, while normal male ranges are 8–35 nmol/liter for testosterone, 10–70 nmol/liter for SHBG, and 1–8 IU/liter for LH. Free testosterone was estimated with Vermeulen's method (23). The total testosterone assay accounts for the majority of variance (>80%) in the free testosterone estimate; detailed analyses of its predictive accuracy have been published elsewhere (24, 25). Serum glucose, high-density lipoprotein and low-density lipoprotein (LDL), total cholesterol, and triglycerides were assayed using a Roche Hitachi 917 analyzer (Roche Diagnostic GmbH, Mannheim, Germany). Serum C-reactive protein (CRP) was measured with a high-sensitivity particle-enhanced immunonephelometry assay using a Dade Behring BN-II analyzer (Dade Behring, Birmingham, UK).

Other measurements

Height (in centimeters), weight (in kilograms), waist and hip circumference (in centimeters), and blood pressure were measured at W1 and W2. Questionnaire data (W1 and W2) and biochemistry (W2) were used to flag dyslipidemia and diabetes. Questionnaire and clinical data (W1 and W2) were used to identify hypertension. Men were asked about tobacco use at both time points. At W1, the greatest transverse and anteroposterior diameter of the abdominal aorta was measured with a Toshiba Capasee ultrasound machine with a 3.75-MHz probe (Toshiba Australia, North Ryde, Australia).

Morbidity and mortality

We assessed morbidity and mortality via the Western Australian Data Linkage System (WADLS), which provides electronic linkage to the state's population health collections (26). WADLS includes records from the death, hospital, and cancer registries and captures separations from all hospitals in the state since 1970. Participants were considered to have preexisting CVD if any hospital record contained diagnosis codes for ischemic heart disease, stroke/transient ischemic attack, abdominal aortic aneurysm, or other cardiovascular diseases (see Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org); if they self-reported these conditions in either questionnaire; or if abdominal aortic diameter at W1 measured 30 mm or more. Cancer diagnoses were identified from the cancer registry. We also constructed a generalized measure of medical comorbidity using Charlson's methodology (27) and adjusted for this in multivariate analyses. Hospital records from 1990 to W2 were used to create this measure.

Cause of death

Primary cause of death was ascertained from WADLS, which contains both the original death certificate, and an ICD-10 coded record generated from these data and other sources by the Australian Bureau of Statistics. At the time of linkage, all deaths occurring in the state before December 31, 2008, were considered to have been recorded in WADLS. We therefore truncated our analysis at this point. For the final year of deaths (2008), Australian Bureau of Statistics coding was not yet available. These deaths were ICD-10 coded by Z.H. and reviewed by B.B.Y. and P.E.N. (who were blinded to biochemical data) and categorized into deaths from cardiovascular disease, respiratory diseases, cancer, and other causes (see Supplemental Table 2 for codes).

Statistical analysis

We used Stata version 11.1 to analyze the data (StataCorp, College Station, TX). Of men providing sera, testosterone and LH were successfully assayed in 4165 and SHBG in 4162. From these, we excluded orchidectomized men, those with prostate cancer, and those receiving antiandrogens, GnRH analogs, or testosterone therapy, leaving 3638 men (including three without SHBG). One man subsequently withdrew, leaving 3637 participants for analysis. We used Pearson's χ^2 test to assess associations in categorical variables between groups and Kruskal-Wallis and Mann-Whitney *U* tests to assess continuous data. A Cox proportional hazards model was used to test associations between sex hormones and all-cause mortality. Because the relationship between sex hormones and mortality appeared curvilinear, hormones were entered into the models as restricted cubic splines. We report hazard ratios at specific points along the spline that may be of interest to the reader; they do not represent division of the data into quantiles but instead show how the hazard function changes across the data. The Schoenfeld residuals were

TABLE 1. Baseline (2001–2004) demographic, biochemical, and clinical characteristics of men by mortality status at end of follow-up

Variable	Alive (n = 3032)	Died from CVD (n = 207)	Died from other causes (n = 398)	*P* value
Age (yr), mean ± SD	76.6 ± 3.4	79.0 ± 4.0	78.9 ± 4.0	<0.001
WHR, mean ± SD	0.97 ± 0.1	0.98 ± 0.1	0.97 ± 0.1	0.071
Total testosterone (nmol/liter), mean ± SD	15.4 ± 5.5	14.8 ± 5.5	15.9 ± 6.3	0.168
Free testosterone (pmol/liter), mean ± SD	280 ± 95	259 ± 91	271 ± 108	<0.001
SHBG (nmol/liter), mean ± SD	41.7 ± 15.9	45.5 ± 21.7	46.4 ± 19.3	<0.001
LH (IU/liter), mean ± SD	5.5 ± 4.5	7.8 ± 9.6	7.0 ± 6.9	<0.001
CRP (mg/liter), mean ± SD	3.5 ± 6.6	4.8 ± 7.0	5.2 ± 8.3	<0.001
Aortic diameter[b] (mm), mean ± SD	22.6 ± 4.6	24.4 ± 6.9	23.8 ± 6.1	<0.001
Hypertension [n (%)]	2319 (76.5)	161 (77.8)	298 (74.9)	0.690
Dyslipidemia [n (%)]	2311 (76.2)	160 (77.3)	275 (69.1)	0.007
Diabetes mellitus [n (%)]	473 (15.6)	40 (19.3)	60 (15.1)	0.336
Smoking status [n (%)]				<0.001
Never smoked	1060 (35.0)	57 (27.5)	99 (24.9)	
Ex-smoker	1832 (60.4)	130 (62.8)	259 (65.1)	
Current smoker	140 (4.6)	20 (9.7)	40 (10.0)	
Charlson's index [n (%)]				<0.001
0	1894 (62.5)	73 (35.3)	192 (48.2)	
1–2	824 (27.2)	71 (34.3)	124 (31.2)	
3–4	223 (7.3)	39 (18.8)	55 (13.8)	
≥5	91 (3.0)	24 (11.6)	27 (6.8)	
Cancer diagnoses[a] [n (%)]				<0.001
0	2308 (76.1)	153 (73.9)	106 (26.6)	
1	588 (19.4)	43 (20.8)	215 (54.0)	
2	102 (3.4)	8 (3.9)	60 (15.1)	
≥3	34 (1.1)	3 (1.4)	17 (4.3)	
Prevalent CVD [n (%)]	1224 (40.4)	151 (73.0)	195 (49.0)	<0.001

P values are for the Kruskal-Wallis test and Pearson's χ^2 test for continuous and categorical data, respectively. WHR, Waist to hip ratio.

[a] Cancer diagnoses include those both before and after baseline.

[b] Measured at W1 (1996–1999).

examined to confirm the proportional hazards assumption. Associations between sex hormones and cause-specific mortality were explored with competing-risks models, as described by Fine and Gray (28). We chose this approach because traditional Cox and Kaplan-Meier models have a key limitation: they assume the event of interest will eventually occur. For example, if, during a study of cancer, a subject died from heart disease (a competing risk), the subject is treated as if they were right censored and could later develop cancer, which is impossible. The Fine and Gray model controls for this bias and yields a sub-hazard ratio (SHR), which is the ratio of hazards associated with the cumulative incidence function (or absolute cause-specific risk) under varying values of a given covariate. The SHR can be interpreted like a hazard ratio. Competing risk was defined as mortality from causes other than the one of interest (*e.g.* death from respiratory disease, cancer, or other non-CVD mortality was the competing risk in the CVD model). Adjustments were made for age, waist to hip ratio, hypertension, dyslipidemia, Charlson's index, smoking, diabetes, prevalent CVD, and number of cancer diagnoses. All tests were two sided, and P values <0.05 were considered significant.

Results

Mean follow-up duration was 5.1 ± 1.3 yr (range, 0.1–7.2 yr), comprising 18,682 person-years. During this time, there were 605 deaths, of which 207 [34.2%; 95% confidence interval (CI) 30.4–38.1%] were due to CVD. The remainder were attributable to cancer (38.2%; 95% CI = 34.3–42.1%; n = 231), respiratory diseases (21.5%; 95% CI = 18.2–24.8%; n = 130), or other causes (12.6%; 95% CI = 9.9–15.2%; n = 76). There were 39 deaths attributable to both cancer and respiratory diseases.

Characteristics of men by status at end of follow-up

Baseline demographic, biochemical, and clinical characteristics of participants are shown in Table 1, stratified by status at end of follow-up. Men who died were older and had a greater burden of medical comorbidity than

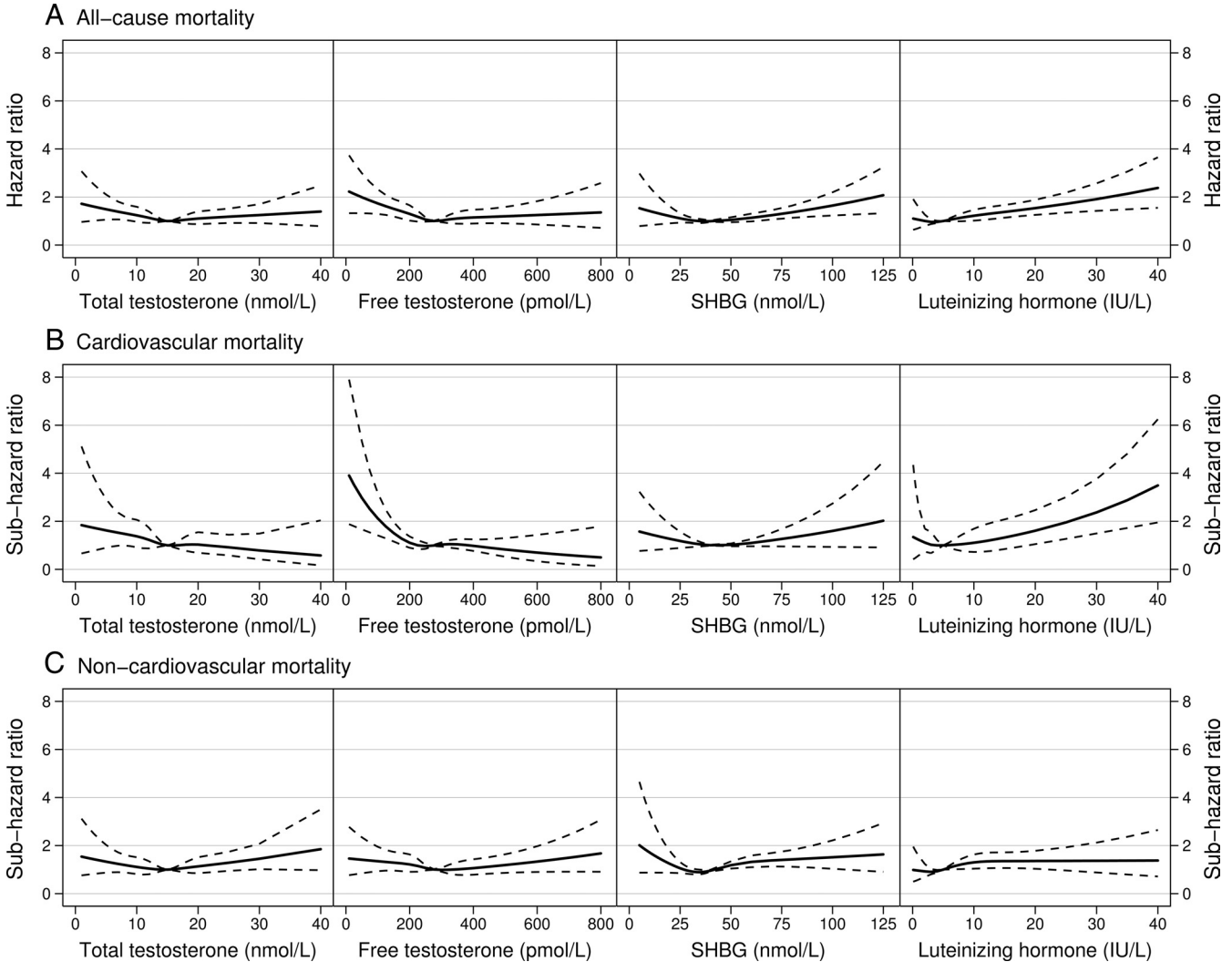

FIG. 1. Univariate Cox and competing-risks proportional hazards models exploring hormone levels and associations with all-cause (A), cardiovascular (B), and noncardiovascular (C) mortality. Sex hormones are entered into the models as restricted cubic splines. Results in A are Cox models; those in B and C are competing-risks models. Reference values for hazard ratios are 15 nmol/liter for total testosterone, 280 pmol/liter for free testosterone, 42 nmol/liter for SHBG, and 5 IU/liter for LH. *Dashed lines* denote 95% CI.

those alive at the end of the study. Free testosterone, SHBG, and LH differed between groups. However, hormonal differences between those who died from CVD or from other causes did not reach statistical significance ($P > 0.05$ for all).

Predictors of all-cause mortality

Sex hormones appeared to exhibit curvilinear relationships with all-cause mortality (Fig. 1). In univariate analyses, low free testosterone and high SHBG were significantly associated with mortality, as was high LH (Table 2). There also appeared to be a curvilinear association between total testosterone and all-cause mortality, but this did not reach statistical significance. After adjustment, low free testosterone and high SHBG and LH continued to be significantly associated with all-cause mortality.

Cardiovascular mortality

We then explored predictors of CVD mortality with competing-risks proportional hazards models (Table 3). Unlike all-cause mortality, total testosterone appeared to have a linear relationship with CVD mortality, with lower levels associated with increased risk. This association did not reach statistical significance, however. Lower free testosterone and higher SHBG and LH levels were significantly associated with reduced survival in both univariate and multivariate models.

Noncardiovascular mortality

Total and free testosterone, and SHBG, appeared to have weak curvilinear relationships with mortality from causes other than CVD (Table 4). Extremes at the low and high ends of these hormones were associated with increased risk, although this reached statistical significance only for SHBG. Higher LH was associated with increased risk. After adjustment, only SHBG remained significantly associated with non-CVD mortality.

We further categorized non-CVD mortality into deaths from cancer, respiratory diseases, and other causes. In adjusted models, higher total testosterone was associated with cancer (SHR = 1.57; 95% CI = 1.17–2.12; for 30 *vs.* 15 nmol/liter), but there was no significant relationship between total testosterone and respiratory diseases ($P = 0.266$) or deaths from other causes ($P = 0.847$). Free testosterone was not significantly associated with any of these outcomes ($P > 0.05$ for all).

To explore why total testosterone was associated with cancer, we classified these deaths into mortality from prostate (n = 18), lung (n = 40), colorectal (n = 26), and other

TABLE 2. Cox proportional hazards models exploring hormone levels and associations with all-cause mortality

	Univariate			Multivariate		
Variable	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Total testosterone (nmol/liter)			0.148			0.223
5	1.49	1.06–2.10		1.27	0.89–1.82	
10	1.24	0.97–1.59		1.29	1.00–1.67	
15	1			1		
20	1.10	0.87–1.40		1.16	0.91–1.47	
30	1.25	0.92–1.70		1.34	0.98–1.83	
Free testosterone (pmol/liter)			0.005			0.024
100	1.73	1.29–2.32		1.62	1.20–2.19	
200	1.30	1.02–1.65		1.34	1.05–1.72	
280	1			1		
300	1.02	0.95–1.10		1.03	0.96–1.11	
400	1.15	0.90–1.47		1.18	0.92–1.52	
SHBG (nmol/liter)			0.008			0.001
20	1.21	0.90–1.63		1.13	0.84–1.52	
30	1.05	0.95–1.15		1.00	0.91–1.10	
40	1			1		
50	1.05	0.93–1.20		1.10	0.97–1.26	
80	1.37	1.13–1.67		1.50	1.23–1.85	
110	1.82	1.28–2.58		2.05	1.42–2.95	
LH (IU/liter)			<0.001			0.001
0.5	1.08	0.67–1.75		0.97	0.59–1.60	
2	1.00	0.80–1.26		0.96	0.75–1.21	
5	1			1		
10	1.22	1.02–1.46		1.17	0.98–1.41	
20	1.54	1.26–1.88		1.45	1.18–1.78	
30	1.91	1.42–2.57		1.78	1.33–2.40	

Sex hormones are entered into the models as restricted cubic splines. Multivariate models are adjusted for age, waist to hip ratio, hypertension, dyslipidemia, diabetes, smoking status, Charlson's weighted comorbidity index, prevalent cardiovascular disease, and number of cancer diagnoses. *P* values are for Wald test. HR, Hazard ratio.

TABLE 3. Competing-risks proportional hazards models exploring hormone levels and associations with cardiovascular mortality

	Univariate			Multivariate		
Variable	**SHR**	**95% CI**	***P* value**	**SHR**	**95% CI**	***P* value**
Total testosterone (nmol/liter)			0.187			0.674
5	1.62	0.90–2.93		1.26	0.68–2.32	
10	1.38	0.92–2.06		1.27	0.84–1.93	
15	1			1		
20	1.03	0.69–1.55		1.07	0.71–1.60	
30	0.79	0.42–1.49		0.85	0.45–1.61	
Free testosterone (pmol/liter)			0.001			0.047
100	2.12	1.41–3.18		1.71	1.12–2.62	
200	1.11	0.90–1.37		1.02	0.82–1.26	
280	1			1		
300	1.03	0.95–1.12		1.05	0.96–1.14	
400	0.98	0.77–1.25		1.03	0.80–1.31	
SHBG (nmol/liter)			0.017			0.017
20	0.95	0.55–1.63		0.91	0.53–1.54	
30	1.11	0.94–1.31		1.07	0.91–1.27	
40	1			1		
50	0.82	0.67–1.01		0.85	0.69–1.06	
80	1.11	0.81–1.52		1.16	0.83–1.61	
110	1.80	1.07–3.01		1.84	1.11–3.05	
LH (IU/liter)			0.001			0.010
0.5	1.29	0.49–3.45		1.31	0.48–3.57	
2	1.11	0.73–1.69		1.07	0.71–1.61	
5	1			1		
10	1.11	0.72–1.69		1.00	0.66–1.54	
20	1.61	1.05–2.47		1.36	0.89–2.09	
30	2.37	1.49–3.76		1.89	1.20–2.98	

Sex hormones are entered into the models as restricted cubic splines. Multivariate models are adjusted for age, waist to hip ratio, hypertension, dyslipidemia, diabetes, smoking status, Charlson's weighted comorbidity index, prevalent cardiovascular disease, and number of cancer diagnoses. *P* values are for Wald test.

cancers (n = 147). In multivariate models (adjusted as per Tables 2–4), higher total testosterone was associated with death from lung cancer [SHR = 1.94 (95% CI = 1.06–3.53) and SHR = 4.05 (95% CI = 2.11–7.75); for 20 and 30 nmol/liter, respectively], as was higher free testosterone (SHR = 1.96; 95% CI = 1.14–3.36; for 400 *vs.* 280 pmol/liter). However, total testosterone was not significantly associated with death from prostate ($P = 0.561$), colorectal ($P = 0.903$), or other cancers ($P = 0.883$). Free testosterone was also not significantly associated with these outcomes ($P > 0.05$ for all).

Men with lung cancer died on average 3.1 ± 1.5 yr after baseline. These men had higher total testosterone than the rest of the sample (19.3 *vs.* 15.4 nmol/liter; $P = 0.005$) but were more likely to smoke (32.5%, n = 13, *vs.* 5.2%, n = 187; $P < 0.001$). With regard to the entire cohort, current smokers also had higher total testosterone than never-smokers (17.0 *vs.* 15.9 nmol/liter; $P = 0.006$) and ex-smokers (15.0 nmol/liter; $P < 0.001$). After excluding current smokers from lung cancer cases, higher free testosterone (SHR = 1.85; 95% CI = 1.01–3.37; for 400 *vs.* 280 pmol/liter) continued to predict lung cancer mortality in univariate analyses (cell counts were too small for multivariate models owing to the small number of cases remaining). However, the association with total testosterone did not retain statistical significance (data not shown). Of men included in sensitivity analyses (n = 27), two never smoked and 25 were ex-smokers. All ex-smokers quit at least 1 yr before testosterone measurement.

Cardiovascular mortality by hypogonadism categories

We recently reported that men with elevated LH were more likely to experience ischemic heart disease (IHD) events (21). To explore whether this association was also present with regard to CVD mortality, we stratified men by gonadal status using established Australian consensus guidelines (29). As shown in Fig. 2, mortality differed between groups, with men with elevated LH at greatest risk.

Assessment of reverse causality

Because illness may affect testosterone levels, we repeated our analyses after excluding 54 men who died within 1 yr of blood collection. After exclusions, free testosterone and LH continued to be significantly associated with CVD mortality, but SHBG did not (data not shown). SHBG remained significantly associated with non-CVD mortality, while total and free testosterone remained sig-

TABLE 4. Competing-risks proportional hazards models exploring hormone levels and associations with mortality from causes other than cardiovascular disease

	Univariate			Multivariate		
Variable	**SHR**	**95% CI**	***P* value**	**SHR**	**95% CI**	***P* value**
Total testosterone (nmol/liter)			0.251			0.117
5	1.33	0.87–2.03		1.16	0.76–1.78	
10	1.11	0.81–1.51		1.16	0.84–1.60	
15	1			1		
20	1.13	0.85–1.51		1.14	0.85–1.52	
30	1.45	1.02–2.07		1.53	1.09–2.13	
Free testosterone (pmol/liter)			0.201			0.200
100	1.35	0.93–1.95		1.29	0.88–1.88	
200	1.22	0.91–1.63		1.26	0.93–1.71	
280	1			1		
300	0.99	0.91–1.07		0.98	0.91–1.07	
400	1.06	0.79–1.43		1.04	0.77–1.41	
SHBG (nmol/liter)			0.018			0.003
20	1.30	0.90–1.87		1.22	0.84–1.77	
30	0.98	0.87–1.10		0.95	0.84–1.07	
40	1			1		
50	1.24	1.05–1.46		1.27	1.08–1.51	
80	1.49	1.15–1.93		1.63	1.25–2.12	
110	1.63	1.03–2.58		1.89	1.19–3.01	
LH (IU/liter)			0.038			0.081
0.5	0.97	0.53–1.77		0.87	0.47–1.62	
2	0.93	0.70–1.23		0.88	0.66–1.19	
5	1			1		
10	1.30	1.04–1.63		1.24	0.99–1.56	
20	1.36	1.03–1.78		1.28	0.97–1.68	
30	1.37	0.88–2.12		1.28	0.84–1.94	

Sex hormones are entered into the models as restricted cubic splines. Multivariate models are adjusted for age, waist to hip ratio, hypertension, dyslipidemia, diabetes, smoking status, Charlson's weighted comorbidity index, prevalent cardiovascular disease, and number of cancer diagnoses. *P* values are for Wald test.

nificantly associated with lung cancer (data not shown). Because inflammatory cytokines can induce resistance to LH, the association between elevated LH and CVD mortality might not be causal and could be explained by the inflammatory milieu associated with atherosclerosis. To investigate this, we performed additional adjustments for CRP, which did not alter the relationship between LH and mortality (data not shown).

Discussion

In this study of older men, low free testosterone and high LH were associated with increased CVD mortality; men with both low free testosterone and high LH were at greatest risk. Elevated SHBG was associated with non-CVD mortality, while higher testosterone was associated with lung cancer. To our knowledge, this is the first time that LH has been associated with CVD mortality in older men and that testosterone has been associated with lung cancer mortality.

Previous observational studies have linked low testosterone to all-cause mortality (12–17). However, findings with regard to cause-specific mortality are mixed. In the Osteoporotic Fractures in Men (MrOS) study, low total testosterone and total estradiol were associated with all-cause mortality (13). Subjects with low levels of both were at highest risk. Low testosterone and estradiol were associated with non-CVD mortality but were not significantly associated with CVD. However, a disparate range of cardiovascular disorders were classified as CVD, which may explain this discrepancy. A linear decrease in CVD event rates across increasing quartiles of total testosterone was reported in the Framingham study, but testosterone was not associated with CVD in adjusted survival analyses (30).

In contrast, low total testosterone was associated with all-cause, CVD, and respiratory mortality in the Rancho Bernardo study (14) and with all-cause and CVD mortality in the EPIC-Norfolk study (16). In the latter analysis, low testosterone was also associated with cancer mortality in univariate analyses, but this was not maintained at a significant level after adjustment.

Low free, but not total, testosterone was associated with all-cause mortality in the Tromsø study (12). Neither hormone was associated with CVD mortality. Total testosterone was also unrelated to all-cause mortality in analyses of middle- to older-aged men in the Massachusetts

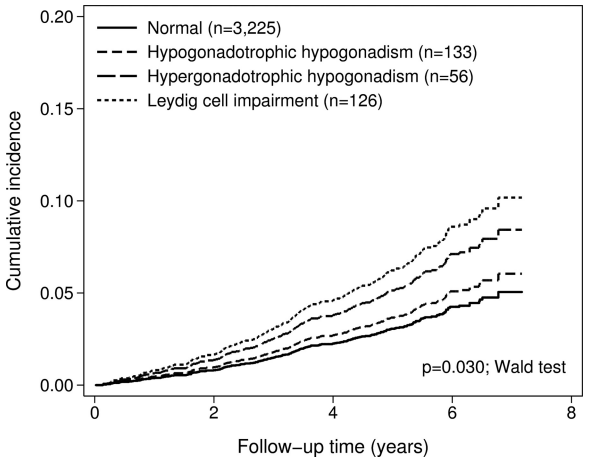

FIG. 2. Cumulative incidence functions for competing-risks proportional hazards model exploring categories of hypogonadism and associations with cardiovascular mortality. Models are adjusted for age, waist to hip ratio, hypertension, dyslipidemia, diabetes, smoking status, Charlson's weighted comorbidity index, prevalent cardiovascular disease, and number of cancer diagnoses. Criteria for categories of gonadal status were as follows: testosterone at least 8 nmol/liter and LH no more than 12 IU/liter, normal gonadal function; testosterone less than 8 nmol/liter and LH no more than 12 IU/liter, hypogonadotropic hypogonadism; testosterone less than 8 nmol/liter and LH more than 12 IU/liter, hypergonadotropic hypogonadism; and testosterone 8–15 nmol/liter and LH more than 12 IU/liter, Leydig cell impairment.

Male Aging Study (20). However, low free testosterone was associated with increased respiratory mortality but decreased ischemic heart disease mortality.

The associations we observed with regard to CVD mortality are consistent with the known effects of androgens on the cardiovascular system. Androgen deficiency is associated with dyslipidemia and abdominal obesity, while testosterone therapy generally decreases LDL and total cholesterol and improves body composition (9, 31). Testosterone stimulates protein synthesis and encourages differentiation of pluripotent cells into myocytes rather than adipocytes (32). Lipid uptake by adipocytes is also inhibited. Testosterone correlates inversely with fibrinogen and suppresses proinflammatory cytokines, which are thought to be involved in the development of insulin resistance and atherosclerosis (31). LDL can penetrate the normal endothelium to lodge in the arterial wall, provoking an inflammatory response and the eventual formation of an atherosclerotic plaque. A continued inflammatory response may compromise plaque stability, resulting in rupture and an acute ischemic event (31). Testosterone also has dose-dependent vasodilatory actions, which are uninhibited by androgen receptor (AR) antagonists (33). These observations suggest androgens have beneficial effects on the vasculature, mediated through genomic and nongenomic mechanisms.

However, a recent testosterone trial in elderly men reported excess cardiovascular events in the treatment group (11). This should be interpreted with caution, owing to the degree of cardiovascular morbidity in the cohort and high testosterone dose received by some men. The majority of trials report no cardiovascular concerns (34). Nevertheless, it raises the question of whether there is a timing effect as observed in trials of hormone therapy in women. Estrogen appears to have vasoprotective effects in young women but harmful effects in older women with established atherosclerosis (35).

Some have argued that gonadotropins are of limited value in the assessment of androgen deficiency in aging men (36), but our data suggest otherwise. Elevated LH strongly predicted CVD mortality after adjustment for medical comorbidity and CRP. Importantly, those with low-normal testosterone but elevated LH also exhibited increased risk. Leydig cell impairment (compensated hypogonadism) has been mooted as a discrete diagnostic category (37), and our results suggest it may have prognostic value. Assessment of androgen deficiency should therefore include measurement of both testosterone and gonadotropins.

With the exception of lung cancer, neither testosterone nor gonadotropins were significantly associated with non-CVD mortality, with only SHBG remaining associated in adjusted analyses. The latter is unsurprising, because SHBG rises strongly with age and medical comorbidity. Low SHBG has been associated with cardiovascular risk factors, including dyslipidemia and metabolic syndrome, and with mortality (38, 39), but SHBG is down-regulated by obesity and hyperinsulinemia, which most likely explains these associations. We found no evidence for a causal relationship between low SHBG and mortality from any cause. In contrast, the association between high testosterone and lung cancer is noteworthy but requires validation elsewhere. Previously, low testosterone has been associated with cancer, which is probably explained by the high prevalence of testicular dysfunction in malignant disease (40). Although we adjusted for smoking, our findings are most likely attributable to residual confounding, because smoking can affect testosterone levels (41). Additionally, reverse causality cannot be dismissed, because both endocrine and nonendocrine tumors can secrete hormones, including LH and estradiol (42). However, a causal role remains plausible.

Men exhibit better resistance to the cellular effects of tobacco-related carcinogens than women but have poorer survival once malignancy is established (43, 44). There are also sex differences in normal lung development, with maturation taking longer in males (45). Sex hormones are thought to underlie these differences (46, 47). The AR is expressed in normal and malignant lung tissue, and testosterone has been shown to alter expression of genes reg-

ulating apoptosis and metabolism in malignant cells *in vitro* (44). Testosterone stimulates growth of small-cell lung cancer cell lines, an effect inhibited by AR antagonists (48). Alternatively, estrogenic mechanisms may be involved. Estradiol stimulates proliferation of human non-small-cell lung cancer cells both *in vitro* and *in vivo* (49), and aromatase expression predicts mortality in women with non-small-cell lung cancer (50). This mechanism might theoretically apply to men, with those with higher testosterone (providing increased substrate for aromatization) at greater risk. However, these speculative hypotheses require further investigation in other large cohort studies.

Strengths of our study include the large, population-based sample, focus on older men, adjustment for competing risks, and near-complete capture of endpoints via electronic record linkage. Limitations include the single blood sample, estimation of free testosterone, and lack of other hormone data, such as estradiol. However, a single measurement of testosterone is considered reliable in the context of large-scale analyses (51). The Vermeulen method has been reported to both overestimate (24) and underestimate (25) free testosterone compared with laboratory measures, but direct measurement of free testosterone was impractical. Nonetheless, estimated free testosterone remains useful in the research context, although its application in other settings should be approached cautiously. We did not have the resources to assay additional hormones. The concept of free testosterone, in which the free portion of testosterone is assumed to be more biologically active than that bound to SHBG and albumin, has been criticized (52). However, our observations with regard to LH suggest that the association between free testosterone and mortality is genuine rather than attributable to artifact. The lack of a statistically significant association between total testosterone and mortality, therefore, may lend support to the free hormone hypothesis or could instead be attributable to our immunoassay. Measurement error may be notable at low levels of testosterone, which would introduce bias in favor of the null hypothesis.

In summary, our results suggest low testosterone predicts CVD mortality but not death from other causes. The association between testosterone and lung cancer is noteworthy and requires further investigation, although it most likely reflects confounding, which is difficult to eliminate in observational studies. The question of whether low testosterone is merely a biomarker for illness or has a causal role in disease remains unresolved. Our data suggest clinical trials should investigate whether preventing androgen deficiency can improve cardiovascular outcomes. Trials should enroll men with elevated LH as well as those with low testosterone. However, given the relatively small associations observed, beneficial effects are likely to be modest and will require large sample sizes to demonstrate. Although some have argued for greater use of testosterone, suggesting it may increase not only quality of life but also lifespan (53), our findings would not support this concept. Use outside of current clinical guidelines would be inconsistent with evidence-based medicine and seems reminiscent of the age-old quest for an elixir of youth.

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