Low Serum Testosterone and Mortality in Older Men

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Context: Declining testosterone levels in elderly men are thought to underlie many of the symptoms and diseases of aging; however, studies demonstrating associations of low testosterone with clinical outcomes are few.

Objective: The objective of the study was to examine the association of endogenous testosterone levels with mortality in older community-dwelling men.

Design, Setting, and Participants: This was a prospective, population-based study of 794 men, aged 50–91 (median 73.6) yr who had serum testosterone measurements at baseline (1984–1987) and were followed for mortality through July 2004.

Main Outcome Measure: All-cause mortality by serum testosterone level was measured.

Results: During an average 11.8-yr follow-up, 538 deaths occurred. Men whose total testosterone levels were in the lowest quartile (<241 ng/dl) were 40% [hazards ratio (HR) 1.40; 95% confidence interval (CI) 1.14–1.71] more likely to die than those with higher levels, independent of age, adiposity, and lifestyle. Additional adjustment for health status markers, lipids, lipoproteins, blood pressure, glycemia, adipocytokines, and estradiol levels had minimal effect on results. The low testosterone-mortality association was also independent of the metabolic syndrome, diabetes, and prevalent cardiovascular disease but was attenuated by adjustment for IL-6 and C-reactive protein. In cause-specific analyses, low testosterone predicted increased risk of cardiovascular (HR 1.38; 95% CI 1.02–1.85) and respiratory disease (HR 2.29; 95% CI 1.25–4.20) mortality but was not significantly related to cancer death (HR 1.34; 95% CI 0.89–2.00). Results were similar for bioavailable testosterone.

Conclusions: Testosterone insufficiency in older men is associated with increased risk of death over the following 20 yr, independent of multiple risk factors and several preexisting health conditions. (*J Clin Endocrinol Metab* 93: 68–75, 2008)

In contrast to the dramatic fall in estrogen levels at the time of menopause in women, testosterone concentrations in men decline gradually with aging. Many adverse aspects of male aging have been attributed to the decrease in endogenous testosterone, stimulating a surge of interest in testosterone therapy for middle-aged and older men. Testosterone sales in the United States increased 20-fold during the 1990s (1). However, solid evidence linking testosterone insufficiency to health-related outcomes in

older men is just beginning to emerge, and even less information is available on testosterone and mortality.

Approximately 30% of men 60 yr old and older are estimated to have low testosterone (2), which is often accompanied by undesirable signs and symptoms such as low bone and muscle mass; increased fat mass (especially central adiposity); low energy; and impaired physical, sexual, and cognitive function. That these complaints have clinical consequences is supported by prospective cohort studies showing that men with low testosterone

Abbreviations: BMI, Body mass index; CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; HR, hazards ratio; LDL, low-density lipoprotein; MMAS, Massachusetts Male Aging Study.

are at increased risk of falls (3); hip fracture (if estradiol is also low) (4); anemia (5); type 2 diabetes (6); depressive illness (7); and, in some studies, Alzheimer's disease (8, 9). Whether these associations translate into reduced survival is less clear.

A small study of men living in a geriatric rehabilitation unit found that those with low testosterone had increased 6-month mortality, compared with men of the same age, and medical morbidity whose testosterone levels were normal (10). Low testosterone levels were also associated with increased mortality over the following 8 yr in a larger retrospective study of male veterans who attended a hospital clinic (11). However, the men in these studies were in poor health, and acute illness is known to reduce testosterone production (12). Two recent population-based studies of comparatively healthy men with longer follow-up (15–16 yr) did not find an association of testosterone with survival (13, 14). However, the men in these studies were relatively young at baseline (mean age early 50s) and the survival effects of testosterone insufficiency may only be apparent late in the lifespan.

We report here the association of serum testosterone with all cause and cause-specific mortality among 794 community-dwelling older men from the Rancho Bernardo Study whose average age was 72 yr at baseline and who were followed for 20 yr.

Subjects and Methods

Study population

The Rancho Bernardo Study is a population-based study of healthy aging in Caucasian residents of a Southern California community. Between 1984 and 1987, 82% (n = 1060) of surviving community-dwelling older male participants attended a research clinic visit. During this visit, information regarding medical history, medication use, physical activity (exercise 3+ times per week, yes/no), alcohol consumption (1+drinks/day *vs.* less or none), and current smoking (yes/no) was obtained using standard questionnaires. Current medication use was validated by examination of pills and prescriptions brought to the clinic for that purpose. The study protocol was approved by the Institutional Review Board of the University of California, San Diego. All participants gave written informed consent.

Of the 1060 men who attended the 1984–1987 clinic visit, 885 (84%) had sufficient stored sera for measurement of testosterone and other sex hormones. Twenty men were excluded for testosterone levels suggestive of surgical or biochemical castration (<90 ng/dl), 53 decedents because death certificates have not been obtained, and 18 for loss to follow-up. The remaining 794 men (75% of the original cohort) are the subject of this report. Compared with those without hormone assays, men with testosterone measurements were slightly younger and less likely to have diabetes but did not differ in terms of weight, body mass index (BMI), waist girth, lifestyle characteristics, or known heart disease.

Clinical measurements

Height, weight, and waist and hip girth were measured in the clinic with participants wearing light clothing and no shoes. BMI (kilograms per square meter) and waist to hip ratio were used as estimates of overall and central adiposity. Systolic blood pressure was measured twice in seated resting subjects using the Hypertension Detection and Follow–up Program protocol (15); the mean of two readings was used in analyses. Weight change, an indicator of health status, was determined by subtracting the participant's weight at the 1984–1987 visit from that obtained at a 1972–1974 visit (99% of participants).

Blood samples were obtained by venipuncture between 0730 and

1100 h after a requested 12-h fast; serum and plasma were separated and frozen at −70 C until first thawed for sex hormone assays in 1992–1994. Hormone levels were measured in the University of California, San Diego, endocrinology research laboratory of S. S. C. Yen. Total testosterone and estradiol levels were measured by RIA after solvent extraction and celite column chromatography; procedural losses were monitored by addition of tritiated standard to each sample before the extraction step. Bioavailable testosterone and estradiol (the non-SHBG bound fractions) were measured by an adaptation of the Tremblay and Dube ammonium-sulfate precipitation method (16). In 2000, plasma C-reactive protein (CRP) and IL-6 were measured on a subset of 614 participants in a university laboratory by an automated, high-sensitivity method (N Latex CRP mono; Dade Behring, Deerfield, IL; sensitivity: 0.2 mg/liter) and a high sensitivity (0.094 pg/ml) ELISA with an alkaline phosphatase signal amplification system (Quantikine HS, human IL-6 immunoassay; R&D Systems, Minneapolis, MN), respectively. In 2004 adiponectin and leptin levels were measured by RIA on twice-thawed serum samples at Linco Diagnostics Laboratory (St. Louis, MO). The sensitivity and intra- and interassay coefficients of variation, respectively, were 3 pg/ml and 6 and 7% for estradiol; 3 pg/ml × percentage free and 6 and 8% for bioavailable estradiol; 20 pg/ml and 4 and 5% for testosterone; 20 pg/ml × percentage free and 7%, and 11% for bioavailable testosterone; 0·8 mg/liter and 6% and 7% for adiponectin; and 0·5 ng/ml and 4% and 5% for leptin. Adiponectin, leptin and sex hormone levels did not vary by years of frozen sample storage.

Fasting plasma total, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol, and triglyceride levels were measured in a Centers for Disease Control and Prevention Certified Lipid Research Clinic Laboratory. Total cholesterol and triglyceride levels were measured by enzymatic techniques using an ABA-200 biochromatic analyzer (Abbott Laboratories, Irving, TX). HDL was measured after precipitation of the other lipoproteins with heparin and manganese chloride. LDL was estimated using the Friedewald formula (17). Plasma glucose levels were measured by the glucose oxidase method, plasma insulin by double-antibody RIA, and serum creatinine by the Jaffe reaction method. Homeostasis model assessment for insulin resistance (HOMA-IR) was used to estimate insulin resistance according to the formula: insulin (milliunits per liter) × glucose (millimoles per liter)]/22.5.

Prevalent conditions and mortality assessment

Information on diabetes and cardiovascular disease (CVD) history was obtained using standard interview questionnaires. Prevalent CVD included angina [by physician diagnosis, Rose questionnaire (18), or angina medication use], doctor-diagnosed myocardial infarction, cardiac revascularization, congestive heart failure, stroke or transient ischemic attack, carotid surgery, peripheral arterial surgery, or physician-diagnosed intermittent claudication. Diabetes was defined by physician diagnosis, fasting plasma glucose 7.0 mmol/liter or greater (126 mg/dl), 2-h postchallenge glucose 11.1 mmol/liter or greater (200 mg/dl), or use of diabetes medication (19). The metabolic syndrome was defined using 2002 National Cholesterol Education Program Adult Treatment Panel III criteria (20). Hypertension was defined as blood pressure 130/85 mm Hg or greater or use of antihypertensive medication.

Participants were followed through July 2004 by annual mailers and periodic clinic visits. Vital status was known for 98% of participants. Death certificates, obtained for 92% of decedents, were classified for underlying cause of death by a certified nosologist using the *International Classification of Diseases, Ninth Revision*. CVD deaths included codes 401–448, cancer deaths codes 140–208, and respiratory disease deaths codes 460–519.

Statistical analysis

Sex hormone, HDL cholesterol, triglycerides, HOMA-IR, IL-6, CRP, leptin, and adiponectin levels were not normally distributed and were natural log transformed for analyses; reported values are geometric means and interquartile ranges. The age-adjusted association of testosterone with baseline characteristics was assessed by partial Pearson cor-

relations for continuous variables and comparing testosterone levels for those with and without each dichotomous characteristic using ANOVA.

The association between baseline testosterone levels and all-cause mortality was determined using Cox proportional hazards regressions; goodness of fit was confirmed by the method by May and Hosmer (21). Total and bioavailable testosterone levels were examined as quartiles based on the entire population and as dichotomous variables comparing the lowest quartile to all higher. Total testosterone was further examined as deciles to determine the optimal level. Three separate regression models were evaluated in mortality analyses: the first adjusted for age, the second added adjustment for BMI and waist to hip ratio, the third added adjustment for lifestyle factors including physical activity (3+ times per week, yes/no), alcohol use (1+ drinks/d *vs.* less or none), and current smoking habit (yes/no). Secondary models added adjustment for testosterone covariates and potential biological mediators. There was no multicollinearity between independent variables in these models, and no significant interactions were found. The proportional odds assumption, assessed visually using log minus log plots, was met by all models.

All *P* presented are two tailed; $P \leq 0.05$ was considered statistically significant. Data were analyzed using SAS (version 9.1; SAS Institute, Cary, NC) and SPSS (version 15.0; SPSS Inc., Chicago, IL).

Results

Baseline characteristics are shown in Tables 1 and 2. The median age of these 794 men was 73.8 yr (range 50–91), 10% had a BMI greater than 30 kg/m^2, and 15% met Adult Treatment Panel III criteria for central obesity (waist girth > 102 cm). Only 11% reported current smoking, half consumed at least one alcohol drink daily, and 84% reported exercising three or more times per week. The median total testosterone level was 300 ng/dl, and 27% of values were below the lower limit (246 ng/dl) of the reference range for young men for the assay used in this study. The median bioavailable testosterone level was 96 ng/dl (no reference range is available).

Testosterone and baseline characteristics

The associations of testosterone levels with baseline characteristics, lifestyle variables, health status markers, and prevalent conditions are presented in Tables 1 and 2. In these elderly men,

TABLE 1. Baseline characteristics and age-adjusted correlations with total testosterone (total T) and bioavailable testosterone (BioT) levels

	Mean	Interquartile range	Total T R[a]	BioT R[a]
Age, yr	71.2	63.6–78.9	0.06	−0.46[b]
Anthropomorphic parameters				
Weight, kg	78.0	70.3–84.8	−0.28[b]	−0.11[c]
BMI, kg/m^2	25.7	23.5–27.4	−0.28[b]	−0.07[d]
Waist to hip ratio	0.91	0.88–0.94	−0.20[b]	−0.05
Cardiovascular risk factors				
Heart rate	60.9	54–67	−0.04	−0.03
SBP, mm Hg	139.7	126–151	−0.11[c]	−0.02
DBP, mm Hg	78.2	72–84	−0.13[b]	0.01
Fasting glucose, mg/dl[e]	102.1	93–109	−0.14[c]	0.02
Fasting insulin, mIU/ml[e,f]	12.0	8.2–16.0	−0.25[b]	−0.10[d]
HOMA-IR[e,f,g]	2.97	2.1–4.2	−0.27[b]	−0.09[d]
Cholesterol, mg/dl				
Total	210.5	186–235	−0.01	0.05
LDL	133.1	111–155	0.07	0.09[d]
HDL[e]	51.2	43–60	0.23[b]	0.03
Triglycerides, mg/dl[e]	105.6	71–148	−0.35[b]	−0.04
Biomarkers				
CRP, mg/liter[e,h]	1.78	0.9–3.4	−0.21[b]	−0.12[c]
IL-6, mg/liter[e,h]	2.63	1.7–3.9	−0.14[c]	−0.13[c]
Adiponectin, mg/liter[e]	9.72	6.8–14.6	0.29[b]	−0.01
Leptin, μg/liter[e]	6.22	4.1–9.0	−0.32[b]	−0.10[c]
Serum creatinine, mg/dl[e]	1.22	1.1–1.4	−0.04	0.03
Sex hormones				
Testosterone, ng/dl[e]	300	242–371		0.53[b]
Biotestosterone, pg/ml[e]	94	78–116	0.53[b]	
Estradiol, pg/ml[e]	19	16–24	0.50[b]	0.35[b]
Bioestradiol, pg/ml[e]	12	10–16	0.25[b]	0.52[b]

SBP, Systolic blood pressure; DBP, diastolic blood pressure.

[a] R is the age-adjusted partial correlation coefficient for each characteristic *vs.* log-transformed total and bioavailable testosterone levels.

[b] $P < 0.001$.

[c] $P < 0.01$.

[d] $P < 0.05$.

[e] Values are geometric means, correlations based on log-transformed variables.

[f] Available on a subset of 556 men.

[g] HOMA-IR.

[h] Available on a subset of 614 men.

TABLE 2. Age-adjusted total and bioavailable testosterone levels in men with and without selected lifestyle variables, health status markers, and prevalent conditions

	Prevalence, %	Total T with/without (ng/dl)	BioT with/without (ng/dl)
Lifestyle			
Current smoking	11	316 / 296	96 / 94
Alcohol use, ≥1 drinks/d	51	291 / 306[a]	94 / 93
Exercise, ≥3 times/wk	85	299 / 294	95 / 91
Health status markers			
10-yr weight loss ≥ 10 lb	23	311 / 294[a]	90 / 95[a]
10-yr weight gain ≥ 10 lb	17	262 / 306[b]	91 / 95
Two or more doctor visits in past year	69	292 / 311[a]	93 / 96
Two or more current medications	31	287 / 303[a]	93 / 94
Prevalent conditions			
Hypertension	75	292 / 317[b]	94 / 93
Metabolic syndrome	18	244 / 311[c]	90 / 95
Diabetes	15	284 / 300	95 / 94
CVD	35	298 / 302	93 / 95

Values are geometric means, analyses based on log-transformed testosterone levels. T, Testosterone; BioT, bioavailable T.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

bioavailable, but not total, testosterone levels were inversely related to age. However, after adjusting for age, total testosterone levels were associated with more risk factors than bioavailable testosterone and the association was generally stronger. Total testosterone was inversely associated with weight, BMI, waist to hip ratio, systolic and diastolic blood pressure, fasting plasma glucose and serum insulin, HOMA-IR, triglycerides, CRP and leptin levels and positively related to HDL cholesterol and adiponectin levels (all $P < 0.01$).

Total testosterone did not differ by smoking habit or exercise but was slightly lower among men who consumed at least one alcohol drink daily, compared with those who drank less or not at all ($P < 0.05$). Levels of total testosterone were higher in men who had lost 10 or more pounds in the past 10 yr ($P < 0.05$) and lower in men who had gained at least 10 pounds ($P < 0.001$),

those who reported two or more visits to the doctor in the past year ($P < 0.05$), and men who were taking two or more medications ($P < 0.05$). Total testosterone did not differ significantly for men with or without CVD or diabetes but was 8% lower for men who had hypertension and 22% lower for men who had the metabolic syndrome, compared with those who did not (both $P < 0.001$). Bioavailable testosterone levels were modestly lower in men who had lost weight ($P < 0.05$) but did not differ by other categorical variables.

Testosterone and mortality

During an average 11.8-yr follow-up, 538 deaths occurred, a mortality rate of 57.5 per 1000 person-years. In age-adjusted analyses, the risk of death was significantly elevated for men in the lowest quartile of the total and bioavailable testosterone dis-

TABLE 3. HRs of total testosterone and bioavailable testosterone for all-cause mortality

Hormone quartile	Median hormone level (ng/dl)	Model 1 HR (95% CI)[a]	Model 2 HR (95% CI)[b]	Model 3 HR (95% CI)[c]
Testosterone (ng/dl)				
Q4 (>370)	436	1.0 (ref)	1.0 (ref)	1.0 (ref)
Q3 (300–370)	331	0.90 (0.71, 1.15)	0.95 (0.74, 1.21)	0.93 (0.73, 1.19)
Q2 (241–299)	273	1.04 (0.82, 1.32)	1.14 (0.89, 1.45)	1.15 (0.90, 1.47)
Q1 (<241)	204	1.29 (1.02, 1.64)	1.42 (1.11, 1.82)	1.44 (1.12, 1.84)
P_{TREND}		0.026	0.003	0.002
Bioavailable T (ng/dl)				
Q4 (>117)	135	1.0 (ref)	1.0 (ref)	1.0 (ref)
Q3 (96–117)	105	0.93 (0.71, 1.22)	0.96 (0.73, 1.27)	0.94 (0.72, 1.24)
Q2 (78–95)	87	1.11 (0.85, 1.44)	1.17 (0.89, 1.53)	1.16 (0.89, 1.52)
Q1 (<78)	65	1.46 (1.12, 1.89)	1.52 (1.17, 1.99)	1.50 (1.15, 1.96)
P_{TREND}		0.001	<0.001	<0.001

Ref, Reference category.

[a] Model 1, adjusted for age.

[b] Model 2, adjusted for age, BMI, and waist to hip ratio.

[c] Model 3, model 2 + alcohol use, current smoking, and exercise.
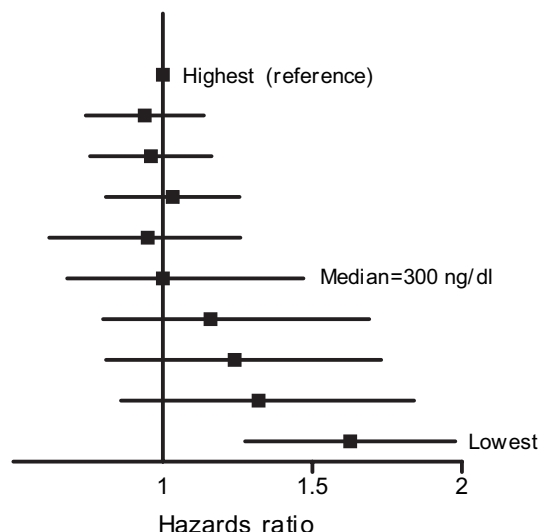
FIG. 1. All-cause mortality according to deciles of total testosterone adjusting for age, BMI, waist to hip ratio, current smoking, alcohol use, and exercise. The *squares* represent point estimates for HRs, the *lines* indicate 95% CIs. The median total testosterone values for deciles 1–10 were 171, 209, 241, 266, 288, 314, 338, 370, 422, and 507 ng/dl, respectively.

tributions, compared with those in the highest quartiles, but did not differ significantly for men in the second and third quartiles (Table 3). In a model adjusting for age, adiposity, and lifestyle choices, the risk of death was 44% higher [hazards ratio (HR) 1.44, 95% confidence interval (CI) 1.12, 1.84)] for men in the lowest quartile of total testosterone relative to the highest, with

similar results for bioavailable testosterone (HR 1.50, 95% CI 1.15, 1.96) (Table 3).

Examination of the association of total testosterone with all-cause mortality by testosterone decile indicated that for men with levels below the median (300 ng/dl), progressively lower levels were associated with a step-wise increase in the risk of death and that there was no additional survival benefit for men in the four highest testosterone deciles, compared with those with midrange levels (Fig. 1).

The influence of testosterone covariates and potential biological mediators was examined by adding variables one by one and in combination to the basic model (age, adiposity, and lifestyle adjusted) and comparing men in the lowest testosterone quartile with those with higher values (Table 4). The strength of the associations of low total and low bioavailable testosterone with increased mortality risk was essentially unchanged after additional adjustment for prevalent diabetes, CVD, or the metabolic syndrome or by exclusion of those with these conditions. Addition of a combination of health status markers, HOMA-IR or adiponectin and leptin, also failed to influence results, as did adjustment for estradiol or bioavailable estradiol levels. Adjustment for the inflammatory markers IL-6 and CRP attenuated the mortality association with low testosterone levels using either total or bioavailable measures. In analyses stratified by CRP level, the HR (95% CI) for low total testosterone was 0.78 (0.52, 1.15) for the 174 men (n = 125 deaths) with CRP 3 mg/dl or greater and 1.66 (1.23, 2.24) for the 440 men (n = 256 deaths) with lower CRP (*P* for interaction = .021).

TABLE 4. HRs of low total testosterone and low bioavailable testosterone for all-cause mortality adjusting for (or excluding) potential covariates and mediators

	Low T HR (95% CI)	Low BioT HR (95% CI)
Lowest quartile *vs.* higher[a]	1.40 (1.14, 1.71)	1.44 (1.19, 1.74)
Plus hypertension[b]	1.39 (1.14, 1.70)	1.45 (1.19, 1.75)
Plus diabetes	1.37 (1.12, 1.69)	1.47 (1.21, 1.79)
Plus CVD	1.35 (1.11, 1.64)	1.44 (1.19, 1.75)
Plus metabolic syndrome	1.30 (1.06, 1.61)	1.45 (1.19, 1.76)
Plus health status markers[c]	1.36 (1.10, 1.67)	1.57 (1.29, 1.92)
Plus HOMA-IR[d]	1.41 (1.08, 1.85)	1.61 (1.24, 2.10)
Plus adiponectin, leptin	1.43 (1.16, 1.77)	1.45 (1.20, 1.77)
Plus CRP, IL-6[e]	1.27 (0.96, 1.57)	1.23 (0.96, 1.58)
Plus estradiol	1.35 (1.08, 1.68)	1.40 (1.15, 1.70)
Plus bioavailable estradiol	1.34 (1.09, 1.66)	1.39 (1.12, 1.71)
Excluding prevalent:		
Diabetes (n = 116)	1.45 (1.15, 1.82)	1.34 (1.09, 1.66)
CVD (n = 274)	1.39 (1.09, 1.77)	1.40 (1.12, 1.76)
Metabolic syndrome (n = 140)	1.43 (1.12, 1.83)	1.32 (1.06, 1.64)

T, Testosterone; BioT, biotestosterone.

[a] Basic model: reference is 241 ng/dl or greater for total testosterone, 78 ng/dl or greater for bioavailable testosterone, adjusted for age, BMI, waist to hip ratio, alcohol use, current smoking, and exercise.

[b] Covariates were added one at a time to the basic model.

[c] The 10-yr weight change, heart rate, number of doctor visits in the past year, number of current medications, and serum creatinine.

[d] HOMA-IR, available on a subset of 556 men: HR for low T = 1.44 (95% CI 1.11, 1.87), HR for low BioT = 1.60 (95% CI 1.23, 2.08) for this subset for the basic model.

[e] CRP and IL-6 available on a subset of 614 men: HR for low T = 1.37 (95% CI 1.08, 1.73), HR for low BioT = 1.40 (95% CI 1.11, 1.77) for this subset for the basic model.

TABLE 5. HRs of low total testosterone and low bioavailable testosterone for cause-specific mortality by years of follow-up

Cause of death	0–20 yr follow-up		5–20 yr follow-up	
	n	HR (95% CI)	n	HR (95% CI)
Low total testosterone[a]				
All-cause	529	1.38 (1.12, 1.69)	409	1.60 (1.27, 2.02)
CVD	264	1.38 (1.02, 1.85)	199	1.73 (1.23, 2.45)
Cancer	127	1.34 (0.89, 2.00)	90	1.22 (0.75, 1.99)
Respiratory disease	54	2.29 (1.25, 4.20)	46	2.67 (1.37, 5.20)
Other	96	1.13 (0.68, 1.88)	83	1.51 (0.89, 2.56)
Low biotestosterone[b]				
All-cause	529	1.44 (1.19, 1.74)	409	1.44 (1.16, 1.80)
CVD	264	1.36 (1.04, 1.79)	199	1.39 (1.01, 1.92)
Cancer	127	1.50 (0.99, 2.26)	90	1.38 (0.82, 2.31)
Respiratory disease	54	1.84 (1.03, 3.28)	46	1.65 (0.86, 3.14)
Other	96	1.43 (0.91, 2.24)	83	1.56 (0.96, 2.53)

Adjusted for age, BMI, waist to hip ratio, alcohol use, current smoking, and exercise.

[a] Reference is total testosterone 241 ng/dl or greater.

[b] Reference is bioavailable testosterone 78 ng/dl or greater.

In age-, adiposity-, and lifestyle-adjusted analyses of cause-specific mortality, low total and bioavailable testosterone were each significantly associated with elevated 20-yr risk of CVD mortality and death due to respiratory disease but not with cancer death or death due to other causes (Table 5). The association of low testosterone with all-cause, CVD, and respiratory disease mortality remained significant after excluding deaths that occurred during the first 5 yr of follow-up.

Discussion

To our knowledge, this is the first prospective population-based study to show that low serum testosterone is associated with increased risk of death in older community-dwelling men. Men with total testosterone levels below the 25th percentile for this population (241 ng/dl) had 40% higher risk of death over the following 20 yr, compared with men with higher endogenous testosterone, independent of age, obesity, and lifestyle choices. These results were similar for the bioavailable fraction of testosterone and were not explained by overall health status.

Testicular function is suppressed in many acute and chronic illnesses, resulting in reduced serum testosterone (12). Low testosterone has been reported in type 2 diabetes, chronic obstructive pulmonary disease, alcoholic liver disease, and chronic renal disease (12). In addition, several cohort studies (22), including this one, have observed lower testosterone in men with the metabolic syndrome, and as many as one in four men with coronary heart disease has testosterone levels in the hypogonadal range (23). Low testosterone could be a marker of preexisting disease and not an independent risk factor for death. However, exclusion of men with diabetes, the metabolic syndrome, or cardiovascular disease had negligible effect on the association of low testosterone with all-cause mortality in this study. Furthermore, the risk estimates for total testosterone were stronger for cardiovascular and respiratory deaths when deaths that occurred during the first 5 yr of follow-up were excluded, suggesting that the testosterone-mortality associa-

tion is not explained by concurrent disease, whether known or hidden.

Research suggests a role for metabolic effects of androgen deficiency in the link between low testosterone and mortality. We, and others, have shown that low testosterone precedes the development of central obesity (24, 25), the metabolic syndrome (26, 27), and diabetes (6, 27, 28) over the following 10–15 yr in nonobese men without these conditions at baseline. In this study, lower total testosterone levels were associated with central obesity and established CVD risk factors including insulin and insulin resistance, glycemia, lipid profile, and blood pressure as well as emerging risk factors such as leptin, adiponectin, IL-6, and CRP. In intervention studies, testosterone therapy improves, and testosterone ablation worsens, central obesity and many of these biomarkers (29), evidence for a causal association. Adjustment for most of these factors had minimal effect on the association of low testosterone with increased mortality. Although a subset analysis suggested some mediation by inflammatory processes, low testosterone was not associated with mortality in men with elevated levels of CRP. Nonetheless, the strong association of androgen deficiency with central obesity and obesity-related factors remains a plausible pathway through which testosterone influences the development of disease and subsequent mortality.

These results differ from those in other prospective studies of testosterone and mortality in relatively healthy community-dwelling men. Testosterone levels were not associated with all-cause mortality in either the Caerphilly Study (13) of 2512 men or the Massachusetts Male Aging Study (MMAS) (14) of 1686 men. The mean duration of follow-up in those studies (16.5 and 15.3 yr, respectively) was somewhat longer than ours (12.4 yr), but the proportion dying was much lower (19 and 31%, respectively, *vs.* 68% for Rancho Bernardo men), reflecting the almost 20 yr older average age of men in the Rancho Bernardo Study. The absent association in the earlier studies may reflect other population differences or hormone assay differences, or the importance of low testosterone for survival may become increasingly important as men age.

The observed low testosterone-mortality association was not

specific to a single etiology. After excluding the first 5 yr of follow-up (to minimize reverse causality by comorbidity), testosterone was strongly associated with deaths due to cardiovascular and respiratory disease. An association of low testosterone with respiratory disease mortality was also reported by the MMAS (14). Unlike the present study, the association in MMAS was observed only with calculated free testosterone and not with total testosterone. In Rancho Bernardo, measured bioavailable testosterone, which includes both the albumin-bound and free testosterone fractions, was significantly associated with respiratory disease death as well as with all-cause mortality; free testosterone was not assayed.

The prevalence of hypogonadism in male populations is not known with certainty, in part due to a lack of consensus on the threshold that should be used to define testosterone insufficiency. Some investigators recommend a total testosterone less than 300 ng/dl; others advocate cut points of 250 or even 200 ng/dl (29). The median testosterone level for the present study (300 ng/dl) was lower than that reported for two population-based cohorts of men of similar age: the Osteoporotic Fractures in Men study of men 65 yr old and older reported a mean total testosterone level of 423 ng/dl (30), and the Health in Men study of men aged 70 yr and older reported a mean of 444 ng/dl (31). Lower testosterone levels in our study are likely due to differences in assay methods: both the Osteoporotic Fractures in Men and Health in Men studies measured testosterone using direct assays, whereas the RIA used in this study was preceded by two purification steps (organic solvent extraction and celite column chromatography), minimizing cross-reactivity with interfering substances (32).

Based on the lower overall mean testosterone, it could be argued that our cut point of 241 ng/dl was too high and overestimates the prevalence of low testosterone. Nonetheless, the 25% of men with values below this threshold were at a 40% increased risk of death over the following 20 yr, suggesting that a sizable proportion of older community-dwelling men may have clinically significant testosterone insufficiency. In a sensitivity analysis, lowering the threshold to 200 ng/dl reduced the proportion of men with low testosterone to 12% and increased the excess mortality risk to 45% ($P = 0.007$). It should be noted that use of these thresholds is arbitrary. There is no current consensus on what constitutes a testosterone insufficient state in older men, or indeed, if one even exists.

There is ongoing debate over the risk-benefit potential of testosterone therapy for men who are not clearly hypogonadal (33, 34). Results of the present study suggest that raising circulating testosterone levels above 300 ng/dl (the median for this population) offers no additional benefit, at least in terms of survival. This, plus the limited availability of information on safety of testosterone replacement, argues against a recommendation for testosterone supplementation to offset effects of male aging in men without documented androgen deficiency.

The present study has some limitations. Results were based on an almost entirely Caucasian middle- to upper-middle-class community and may not apply to other ethnic and socioeconomic groups. Repeat sampling of men with an initially low testosterone level is recommended to confirm androgen deficiency (35), but this and other epidemiological studies were based on a single measurement of testosterone. However, misclassification of men with low testosterone would be expected to underestimate, not cause, asso-

ciations. In addition, blood for testosterone samples was obtained in the morning from fasting men, minimizing any diurnal variation still present in this age group, and sampling at a single time point fairly reliably reflects mean annual testosterone levels for older men (36). Although it is possible that testosterone exerts its benefit by conversion to estradiol, the addition of estradiol to these analyses did not materially change the results, and estradiol was less strongly associated with CVD risk factors than testosterone (data not shown).

In summary, in this cohort, older men with low levels of circulating total testosterone had a 40% increased risk of death over the following 20 yr, compared with men with normal testosterone, independent of age, adiposity, and lifestyle. This association was not explained by preexisting disease and was not specific to a single cause of death. Testosterone levels above the median for this population did not confer any additional survival benefit, offering no support for widespread testosterone therapy for aging men. Randomized, placebo-controlled trials are necessary to determine whether physiological testosterone replacement can safely extend the quality and duration of life for older men with well-documented testosterone insufficiency.

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