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High Serum Testosterone Is Associated With Reduced Risk of Cardiovascular Events in Elderly Men

The MrOS (Osteoporotic Fractures in Men) Study in Sweden

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Objectives We tested the hypothesis that serum total testosterone and sex hormone–binding globulin (SHBG) levels predict cardiovascular (CV) events in community-dwelling elderly men.

Background Low serum testosterone is associated with increased adiposity, an adverse metabolic risk profile, and atherosclerosis. However, few prospective studies have demonstrated a protective link between endogenous testosterone and CV events. Polymorphisms in the SHBG gene are associated with risk of type 2 diabetes, but few studies have addressed SHBG as a predictor of CV events.

Methods We used gas chromatography/mass spectrometry to analyze baseline levels of testosterone in the prospective population-based MrOS (Osteoporotic Fractures in Men) Sweden study (2,416 men, age 69 to 81 years). SHBG was measured by immunoradiometric assay. CV clinical outcomes were obtained from central Swedish registers.

Results During a median 5-year follow-up, 485 CV events occurred. Both total testosterone and SHBG levels were inversely associated with the risk of CV events (trend over quartiles: $p = 0.009$ and $p = 0.012$, respectively). Men in the highest quartile of testosterone (≥550 ng/dl) had a lower risk of CV events compared with men in the 3 lower quartiles (hazard ratio: 0.70, 95% confidence interval: 0.56 to 0.88). This association remained after adjustment for traditional CV risk factors and was not materially changed in analyses excluding men with known CV disease at baseline (hazard ratio: 0.71, 95% confidence interval: 0.53 to 0.95). In models that included both testosterone and SHBG, testosterone but not SHBG predicted CV risk.

Conclusions High serum testosterone predicted a reduced 5-year risk of CV events in elderly men. (J Am Coll Cardiol 2011; 58:1674–81) © 2011 by the American College of Cardiology Foundation

Testosterone has important metabolic actions in men, affecting body composition and exerting direct effects on insulin sensitivity and lipid metabolism (1). In accordance, low serum testosterone is associated with increased adiposity, an adverse metabolic risk profile, and atherosclerosis (1,2). Recently, much interest has focused on testosterone supplementation in elderly men (3), and some studies support the beneficial metabolic effects of testosterone supplementation in men with low testosterone levels (4). However, a recent clinical trial reported that transdermal testosterone therapy increased the risk of cardiovascular (CV) events (5).

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Although most cross-sectional studies have found lower testosterone levels in patients with coronary heart disease (CHD) (6), population-based prospective studies report conflicting results regarding the association between total testosterone and CV mortality (7–13). Few of these studies addressed a possible link between testosterone levels and CV events other than mortality; the Health in Men Study (14) found that both low total testosterone and free testosterone were associated with an increased risk of incident stroke, but other studies (13,15,16) reported no association between testosterone levels and CV events. Given the conflicting results of the relatively few studies addressing the association between endogenous testosterone and CV events in men, there is a need for large epidemiologic studies with complete follow-up of CV endpoints, in which serum testosterone levels are assayed by state-of-the-art methodology.

Sex hormone-binding globulin (SHBG) is an important determinant of total testosterone levels (3,17). Insulin resistance and obesity are associated with low SHBG (1), and it is generally accepted that insulin inhibits the hepatic production of SHBG (1). Interestingly, a recent study showed that polymorphisms in the SHBG gene, affecting serum levels of SHBG, are prospectively associated with the risk of type 2 diabetes, suggesting a causal role for low SHBG in cardiometabolic risk (18). However, studies that addressed SHBG as a predictor of CV events report inconsistent results, and most lack data on concurrent testosterone levels (11,12,14,19,20).

We therefore tested the hypothesis that serum testosterone, assessed by gas chromatography/mass spectrometry, and/or SHBG levels predict CV events in a prospective study of community-dwelling elderly men.

Methods

Study population. The multicenter MrOS (Osteoporotic Fractures in Men) study includes older men in Sweden, Hong Kong, and the United States. In Sweden, study participants (men age 69 to 81 years) were randomly selected from national population registers (21). Eligibility for study participation required the ability to walk unassisted by another person, no bilateral hip prosthesis, the ability to provide self-reported data, and the ability to understand and sign an informed consent; 45% of those contacted participated in the MrOS study in Sweden (n = 3,014), which includes cohorts in 3 cities: Malmö (n = 1,005), Göteborg (n = 1,010), and Uppsala (n = 999). The study was approved by the ethics committees at Göteborg University, Lund University, and Uppsala University.

We investigated the associations between total and free serum testosterone and SHBG with CV events in the Swedish MrOS cohort. Serum samples were drawn in the morning (before 10 AM; 69% of the cohort) or around noon (between 10 AM and 3 PM, average 1 PM; 31%). Serum for assessment of testosterone by gas chromatography/mass spectrometry was available for 2,639 men (99% of the participants in the Göteborg cohort, 96% in the Malmö cohort, and 68% in the Uppsala cohort). Of these, 223 participants were excluded for the following reasons: treatment with testosterone, 5α-reductase inhibitors, gonadotropin-releasing hormone agonists, or antiandrogens or a history of surgical castration. This left 2,416 men in the present study sample.

Abbreviations and Acronyms

Apo = apolipoprotein
BMI = body mass index
CHD = coronary heart disease
CI = confidence interval
CV = cardiovascular
HR = hazard ratio
ICD = International Classification of Diseases
SHBG = sex hormone-binding globulin

Assessment of covariates. We used a standardized questionnaire (22) to gather information about smoking habits and physical activity as well as self-reported medical diagnoses (hypertension, diabetes, stroke, myocardial infarction, or angina pectoris). We defined previous CHD as a history of myocardial infarction and/or angina pectoris and CV disease as a history of stroke or CHD. Hypertension was defined as hypertension diagnosis with either self-reported antihypertensive treatment or a measured systolic blood pressure of ≥140 mm Hg (supine blood pressure, measured after a 10-min rest). Standard equipment was used to measure height and weight; body mass index (BMI) was calculated as kg/m^2 (weight [kg]/height $[m]^2$). Apolipoprotein (Apo) B and ApoA1 were determined by immunoprecipitation enhanced by polyethylene glycol at 340 nm (Thermo Fisher Scientific, Vantaa, Finland); analyses were performed on a Konelab 20 autoanalyzer (Thermo Fisher Scientific), and the interassay coefficient of variation was <5%.

Serum sex steroids and SHBG. All samples were frozen at −80°C and shipped on dry ice to 1 laboratory. A validated gas chromatography/mass spectrometry system (23–25) was used to measure testosterone (detection limit = 0.05 ng/ml; intra-assay coefficient of variation, 2.9%; interassay coefficient of variation, 3.4%) and estradiol (detection limit = 2.00 pg/ml; intra-assay coefficient of variation, 1.5%, interassay coefficient of variation, 2.7%) in baseline serum samples. The analytes and the internal standard were detected using an HP5973 quadruple mass spectrometer equipped with a chemical ionization source (Agilent Technologies, Santa Clara, California). To measure serum SHBG, we used an immunoradiometric assay (Orion Diagnostics, Espoo, Finland; limit of detection, 1.3 nmol/l; intra-assay coefficient of variation, 3%; interassay coefficient of variation, 7%; reference ranges for apparently healthy adult men provided by the manufacturer were 16 to 61 nmol/l). We calculated free testosterone according to the method described by Vermeulen et al. (26) and van den Beld et al. (27), taking concentrations of total testosterone and

SHBG into account and assuming a fixed albumin concentration (43 g/l).

Study outcomes. Follow-up time was recorded as the period between the baseline visit (in the period 2001 to 2004) and date of death, CV event, or last data collection (December 31, 2008). Cause of death data were collected from the Swedish Cause of Death Register, held by the National Board of Health and Welfare in Sweden, in which all deaths in Sweden are registered with International Classification of Diseases (ICD) codes based on the information from death certificates. Data were collected from this register from study start until December 31, 2005, and from evaluation of copies of death certificates for deaths occurring after this date up to 2008. Based on the information from the register/death certificate, the underlying cause of death was determined for each participant and classified as CHD (ICD-10 codes I20 to I25), stroke (ICD-10 codes I60 to I64), or other. Data on hospitalization for first acute myocardial infarction (ICD-10 codes I21 to I23), unstable angina (ICD-10 codes I20.0 and I24), revascularization procedure (surgery code FN), stroke (ICD-10 codes I60 to I64), or transient ischemic attack (ICD-10 code G45) were collected from the Swedish Hospital Discharge Register between the baseline date and December 31, 2008. The combination of data from the Swedish Cause of Death Registry and the Swedish Hospital Discharge Register is an efficient, validated alternative to hospital discharge notes and death certificates for both CHD and stroke (28). Major CV events were defined as a composite endpoint of CHD events (hospitalization for acute myocardial infarction, unstable angina or revascularization, or death from CHD) and cerebrovascular events (hospitalization for stroke or transient ischemic attack, or death from stroke).

Statistical analysis. We used Cox proportional hazards regression to analyze the associations between serum testosterone, SHBG, and CV outcomes. In predefined analyses, total and free testosterone, SHBG, and estradiol were examined as quartiles based on distribution in the entire study population. Based on the observed distribution, testosterone was further examined as a dichotomous variable comparing quartile 4 with quartiles 1 to 3. All estimates were adjusted for age, morning sample (yes/no), and for MrOS study site. Further adjustments were made for BMI (log transformed), ApoB/A1 ratio, physical activity (kilometers walked per day, entered as quartiles because of a non-normal distribution), and 3 dichotomous variables: current smoking, diabetes, and hypertension. Hazard ratios (HRs) were computed for the total cohort and the cohort without baseline history of CV disease. To test whether the associations varied by age, we tested the interaction terms age · testosterone (entered as a dichotomous variable, quartile 4 or quartiles 1 to 3) in the Cox regression model. Unadjusted Kaplan-Meier survival curves illustrated the association between testosterone levels and CV events as well as CHD events and cerebrovascular events, and the log-rank test assessed statistical significance. The baseline frequencies of smoking, diabetes, hypertension, and history of CV disease across testosterone quartiles were tested by the chi-square test (trend across quartiles and quartile 4 vs. quartiles 1 to 3). For continuous baseline variables (age, BMI, ApoB/A1 ratio, physical activity, and hormone levels), the corresponding analyses were performed by linear regression and *t* test. We performed statistical analyses using SPSS for Windows version 18.0 (SPSS Inc., Chicago, Illinois).

Results

The baseline characteristics of the whole cohort as well as trends across quartiles of serum total testosterone levels are shown in Table 1. With increasing levels of testosterone, BMI, ApoB/A1 ratio, and the prevalence of diabetes and hypertension decreased, whereas physical activity increased. Smoking was more common among those with the highest testosterone levels. Prevalent CV disease was inversely

Table 1 Baseline Characteristics of the Whole Sample and by Quartile Group of Serum Testosterone

Variable	Whole Sample (n = 2,416)	Testosterone Quartile 1 (n = 604)	2 (n = 610)	3 (n = 596)	4 (n = 606)	p Value* Trend Across Quartiles	p Value† Q4 vs. Q1–3
Age, yrs	75.4 ± 3.2	75.5 ± 3.2	75.5 ± 3.1	75.4 ± 3.1	75.3 ± 3.3	0.18	0.18
BMI, kg/m^2	26.4 ± 3.6	28.1 ± 3.9	26.7 ± 3.4	25.8 ± 3.1	24.9 ± 3.0	<0.001	<0.001
ApoB/A1 ratio	0.72 ± 0.20	0.75 ± 0.20	0.74 ± 0.20	0.72 ± 0.19	0.68 ± 0.21	<0.001	<0.001
Physical activity, km/day	3.9 ± 3.1	3.4 ± 2.7	3.9 ± 3.1	4.1 ± 3.3	4.2 ± 3.3	<0.001	0.003
Current smoking, %	8.4	7.8	7.4	7.6	10.9	0.058	0.010
Diabetes, %	9.4	15.8	10.8	5.9	5.3	<0.001	<0.001
Hypertension, %	33.7	41.2	37.5	33.0	25.3	<0.001	<0.001
History of CV disease, %	26.2	31.7	31.0	25.4	17.1	<0.001	<0.001
Testosterone, ng/dl	454 ± 173	255 ± 74	389 ± 28	491 ± 31	680 ± 127	—	—
SHBG, nmol/l	44 ± 22	32 ± 18	39 ± 15	45 ± 18	59 ± 27	<0.001	<0.001
Free testosterone, pg/ml	81 ± 31	54 ± 20	74 ± 16	89 ± 22	109 ± 34	<0.001	<0.001
Estradiol, pg/ml	21 ± 7	17 ± 7	20 ± 6	22 ± 6	26 ± 8	<0.001	<0.001

Values are mean ± SD or %. *p value indicates significance for trend across quartiles of testosterone. †p value indicates significance between subjects in quartiles 1 to 3 and quartile 4 of serum testosterone. ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; BMI = body mass index; CV = cardiovascular; Q = quartile; SHBG = sex hormone–binding globulin.

associated with testosterone levels at baseline; a history of CV disease was almost twice as common among participants in testosterone quartiles 1 and 2 compared with those in the highest quartile of testosterone.

The median follow-up time for major CV events was 5.1 years (11,605 person-years). Except for 3 participants who moved abroad, there was no loss to follow-up in the study. During the 5-year follow-up, 485 participants experienced a major CV event (rate of 4.2 per 100 person-years at risk).

Pre-specified analyses based on quartiles of hormone levels revealed an inverse association between quartiles of both total testosterone and SHBG and any major CV event (Table 2), whereas the association between free testosterone and CV risk was not statistically significant (p = 0.061). Using men in quartile 1 of total testosterone levels as a reference, the risk of experiencing a major CV event decreased in men in quartile 4, whereas the risk in the individual quartiles 2 and 3 was similar to that of quartile 1. We therefore pooled quartiles 1 to 3 of total testosterone in subsequent analyses. As shown in Table 2, men in the fourth quartile of testosterone were at a lower risk of CV events compared with men in the pooled quartiles 1 to 3 (p = 0.002). The risk of major CV events across SHBG quartiles displayed a different pattern; men in both individual quartiles 3 and 4 were at lower risk of a major CV event compared with men in quartile 1. In similar analyses, there was no association between estradiol levels and CV risk (Table 2).

Table 2 HRs for Major CV Events Across Quartiles of Serum Testosterone, SHBG, Free Testosterone, and Estradiol

	No. of Major CV Events/ No. at Risk	HR (95% CI)	p Value
Testosterone, ng/dl			
Q1 (≤340)	128/604	1.00 (referent)	
Q2 (341–438)	134/610	1.02 (0.80–1.30)	0.88
Q3 (439–549)	128/596	0.96 (0.75–1.23)	0.74
Q4 (≥550)	95/606	0.71 (0.54-0.93)	0.013
Per-quartile increase		0.90 (0.83–0.97)	0.009
Q4 vs. Q1–3		0.70 (0.56–0.88)	0.002
Q4 vs. Q1–3, adjusted for SHBG*		0.75 (0.59–0.96)	0.024
SHBG, nmol/l			
Q1 (≤29.2)	144/599	1.00 (referent)	
Q2 (29.3–39.4)	125/599	0.86 (0.68–1.10)	0.22
Q3 (39.5–53.2)	103/600	0.71 (0.55–0.93)	0.011
Q4 (≥53.2)	110/599	0.77 (0.60–1.00)	0.050†
Per-quartile increase		0.90 (0.83–0.98)	0.012
Per-quartile increase, adjusted for testosterone‡		0.94 (0.86–1.03)	0.17
Free testosterone, pg/ml			
Q1 (≤62.2)	129/599	1.00 (referent)	
Q2 (62.4–78.9)	123/599	0.95 (0.74–1.22)	0.68
Q3 (78.9–98.0)	118/600	0.84 (0.65–1.09)	0.18
Q4 (≥98.2)	112/599	0.84 (0.64–1.10)	0.20
Per-quartile increase		0.92 (0.85–1.00)	0.061
Estradiol, pg/ml			
Q1 (≤16.1)	123/605	1.00 (referent)	
Q2 (16.1–20.2)	116/601	0.89 (0.69–1.15)	0.37
Q3 (20.3–25.1)	129/606	0.98 (0.77–1.27)	0.90
Q4 (≥25.1)	117/604	0.90 (0.70–1.17)	0.43
Per-quartile increase		0.98 (0.91–1.07)	0.70

Values are adjusted for age, Osteoporotic Fractures in Men study site, and morning sample. *SHBG entered as quartiles. †p < 0.05. ‡Testosterone entered as Q4 versus Q1 to Q3.
CI = confidence interval; HR = hazard ratio; other abbreviations as in Table 1.

Because of the biological interplay between testosterone and SHBG and because both low total testosterone and SHBG levels were associated with CV risk, we next studied the impact of adjusting the testosterone-CV event association for SHBG and vice versa. The association between high testosterone levels and reduced risk of any major CV event remained after adjusting for the SHBG quartile (Table 2); a similar result was obtained if SHBG was entered as below/above the median level (HR: 0.75, 95% CI: 0.59 to 0.96; p = 0.021). In contrast, the association between SHBG and CV risk was no longer statistically significant after adjustment for testosterone levels entered either as quartile 4 versus quartiles 1 to 3 (Table 2) or as individual quartiles (HR: 0.94, 95% CI: 0.85 to 1.03; p = 0.17).

To study the potential modifying effect of age with the association between higher testosterone levels and reduced CV risk, we tested the interaction term age · testosterone in the Cox regression model; the result (p = 0.51) indicated no significant interaction with age (data not shown).

We also calculated the HRs for CV disease outcomes by the highest quartile of testosterone in the whole cohort and the cohort without baseline CV disease. As shown in Figures 1A and 1B, the HR for a major CV event limited to the 1,777 men without baseline CV disease was 0.71 (95% CI: 0.53 to 0.95; p = 0.021), similar to the HR for the total cohort (0.70, 95% CI: 0.56 to 0.88; p = 0.002), despite the smaller number of major CV events (n = 260 in the 1,777 men at risk).

In exploratory post hoc analyses, we examined whether testosterone was associated with the risk of a CHD or a cerebrovascular event. The numbers of CHD events per testosterone quartile were 80 in quartile 1, 80 in quartile 2, 82 in quartile 3, and 60 in quartile 4. The corresponding numbers for cerebrovascular events were 56, 66, 57, and 46, respectively. In analyses for CHD outcomes, high testosterone levels were inversely associated with CHD risk in the total cohort (quartile 4 vs. quartiles 1 to 3 of testosterone, HR: 0.72, 95% CI: 0.54 to 0.96, p = 0.025) (Fig. 1C). After excluding participants with previous CHD (532 participants with 137 events), the point estimate for CHD risk was similar (HR: 0.75, 95% CI: 0.52 to 1.09), but the association was no longer statistically different (Fig. 1D). There was a nonsignificant trend for the association of high testosterone levels with cerebrovascular events in the whole cohort (HR: 0.76, 95% CI: 0.55 to 1.05, p = 0.091) (Fig. 1E), which was similar after exclusion of 155 participants with 34 previous stroke events (HR: 0.72, 95% CI: 0.50 to 1.02, p = 0.063) (Fig. 1F).

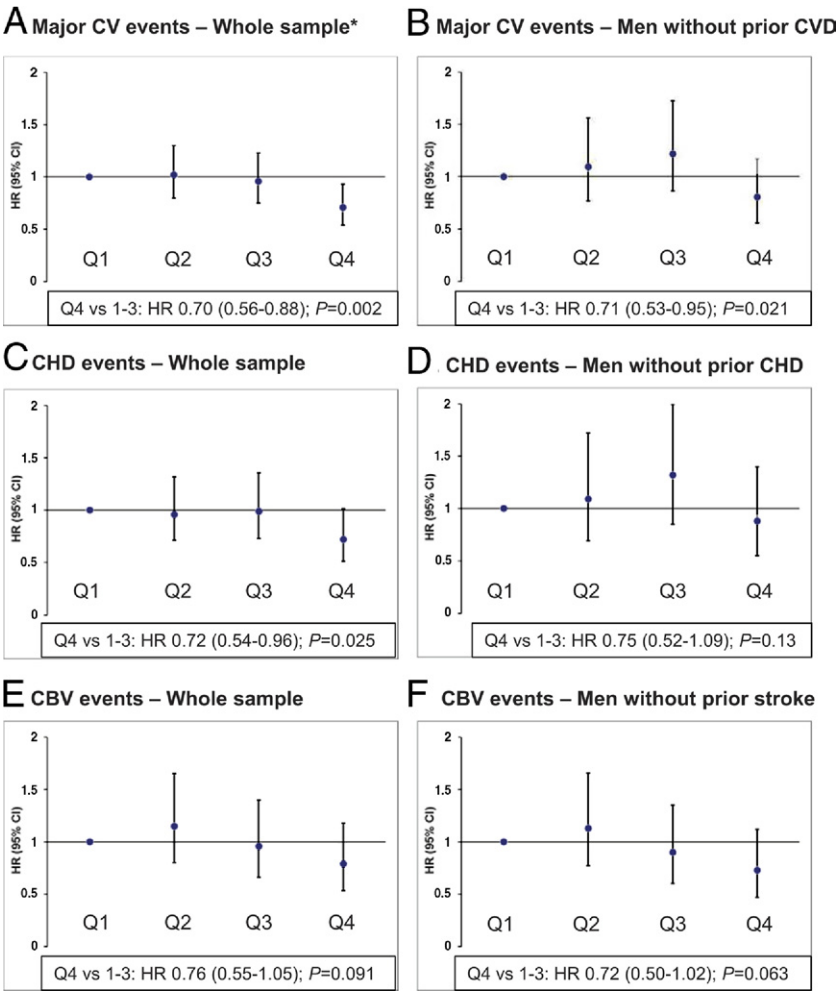

Figure 1 **HRs for CV Events by Quartile of Total Testosterone**

Forest plots showing hazard ratios (HRs) and 95% confidence intervals (CIs) by quartile (Q) of total testosterone levels for major cardiovascular (CV) events **(A, B)**, coronary heart disease (CHD) events **(C, D)**, and cerebrovascular (CBV) events **(E, F)**, in the whole cohort **(A, C, E)** and in subjects without previous disease **(B, D, F)**. Data are adjusted for age, Osteoporotic Fractures in Men study site, and morning sample. *Data from Table 2.

Kaplan-Meier curves of CV event-free survival stratified by total testosterone level also illustrate that men in the highest quartile of testosterone had lower risk of a major CV event (Fig. 2A) (log-rank test, p = 0.002). In corresponding Kaplan-Meier models for CHD and cerebrovascular events, CHD events (log-rank test, p = 0.026), but not cerebrovascular events (p = 0.092), were significantly associated with high testosterone levels (Figs. 2B and 2C).

Because testosterone was associated with CV risk even after adjustment for SHBG levels, and not vice versa, we performed sensitivity analyses for the association between testosterone levels and CV risk, including analyses that excluded participants with a follow-up time of 2.6 years or less (one-half of the median follow-up time), to reduce the potential effect of subclinical or unrecognized disease. CV risk results were unchanged after this exclusion (Table 3). Further, the association between high testosterone and low CV risk was attenuated, but remained statistically significant after adjustment for the traditional CV risk factors shown in Table 3.

Discussion

In this prospective population-based study of elderly Swedish men followed for 5 years, both serum total testosterone and SHBG levels were inversely associated with the risk of CV events. In analyses that included both testosterone and SHBG, high testosterone but not SHBG predicted reduced fatal and nonfatal CV events. Men in the highest quartile of testosterone had lower risk of CV events compared with men in the 3 lower quartiles, and this association remained after adjustment for traditional CV risk factors and after excluding men with prevalent CV disease.

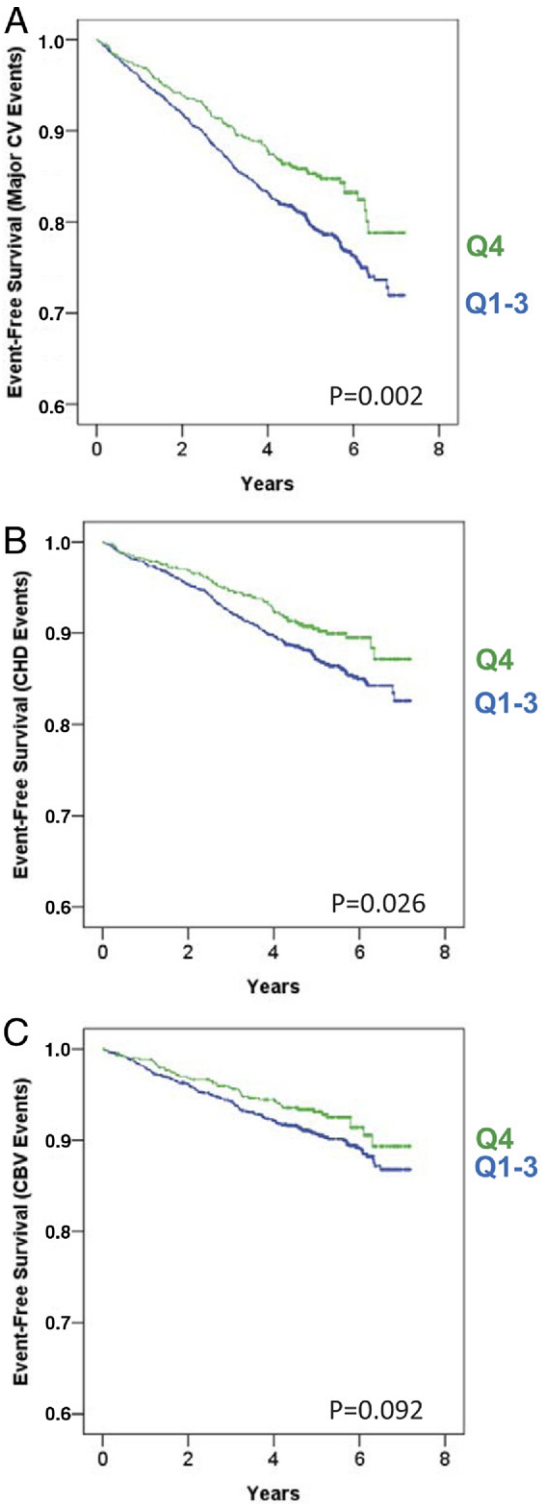

Figure 2 **Kaplan-Meier Plots by Testosterone Levels**

Kaplan-Meier curves of event-free survival by serum testosterone for major CV events **(A)** CHD events **(B)**, and CBV events **(C)**. In quartile 4 of serum testosterone **(green lines)** or quartiles 1 to 3 **(blue lines)**. p value assessed by log-rank test. Abbreviations as in Figure 1.

In a recent small controlled trial of older men selected for impaired physical activity, relatively large doses of exogenous testosterone were associated with an increased risk of adverse CV events (5). This raised concerns about the benefit and safety of testosterone as a cardioprotective agent in older men. In contrast, a recent systematic review and meta-analysis of testosterone trials found no similar harm (29). The data presented here suggest that high endogenous testosterone levels (the highest quartile) are associated with reduced, rather than increased, CV risk in elderly men.

Previous cohort studies of the association between serum testosterone levels and CV events found contradictory results. Large population-based prospective studies reported both an inverse association (Rancho Bernardo [7], European Investigation into Cancer-Norfolk [8], Study of Health in Pomerania [9], Caerphilly [10]) or no association (Massachusetts Male Aging Study [11], National Health And Nutrition Examination Survey [12], Tromsö [13]) between total testosterone and CV disease mortality. Recently, low testosterone levels were shown to predict an increased risk of combined fatal and nonfatal stroke in community-dwelling men with a median age of 76 years (14). There was a nonsignificant trend toward an inverse association between serum testosterone and incident CHD events in the Caerphilly study (10), and other large population-based studies addressing testosterone as a predictor of CV events (Rancho Bernardo [15], Framingham [16], Tromsö [13]) show no association. Thus, our findings of a significant inverse association between testosterone levels and risk of combined fatal and nonfatal CV events in men support and extend previous work showing an association between testosterone and CV mortality (7–10,14). Our study differs from previous studies that all used immunoassay-based hormone assay methods; ours is the first study addressing the association between CV events and serum testosterone assessed by a mass spectrometry–based technique, which provides more accurate assessment of testosterone than immunoassay-based techniques (30).

In the present study, testosterone levels in the highest quartile were associated with reduced CV risk compared with lower levels (quartiles 1 to 3). It is biologically plausible that serum testosterone less than a certain serum level confers increased cardiometabolic risk. Adverse effects of testosterone deficiency on, for example, body composition, insulin sensitivity, and systemic inflammation, may mediate such an effect (1,6). This notion is further supported by the fact that androgen-deprivation therapy in prostate cancer patients increases CV risk (31). In line with previous studies (6), we found that men with high serum testosterone levels had lower BMI and ApoB/A1 ratio, were more physically active, and had a lower frequency of reported diabetes, hypertension, and prevalent CV disease. Although testosterone is associated with an adverse metabolic risk profile, the association between testosterone and risk of CV events remained statistically significant, albeit slightly attenuated,

Table 3 HRs of Testosterone (Q4 vs. Pooled Q1 to Q3) for Major CV Events, First Years of Follow-Up Excluded and Adjustment for Covariates

	No. of Events/ No. at Risk	Testosterone Q4 vs. Q1–Q3 HR (95% CI)	p Value
*Whole sample, adjusted for age**	*485/2,416*	*0.70 (0.56–0.88)*	*0.002*
Excluding first 2.6 yrs† of follow-up, adjusted for age*	252/2,126	0.71 (0.52–0.96)	0.029
Whole sample, adjusted for CV risk factors‡	454/2,267	0.77 (0.60–0.98)	0.032

Values in *italics* are from Table 2. *Adjusted for age, Osteoporotic Fractures in Men study site, and morning sample. †One-half of the median follow-up time 5.1 years. ‡Adjusted for age, Osteoporotic Fractures in Men study site, morning sample, BMI, current smoking, hypertension, diabetes, ApoB/A1 ratio, and physical activity.

Abbreviations as in Tables 1 and 2.

after adjustment for traditional risk factors for CV disease. This may suggest that other mechanisms, such as endothelial regeneration, could be important (32).

Although there are several possible mechanisms by which testosterone potentially reduces CV risk, it is important to note that any severe illness can suppress testosterone production (3,33). Thus, any acute or chronic illness may concomitantly reduce testosterone production and increase the risk of a CV event. Because the elderly are more likely than younger adults to have CV or other diseases, a high testosterone level in elderly men may be a sign of good general health and thereby associated with reduced risk of CV events. However, the absence of statistical evidence of an interaction with age does not support this thesis, but the age range was fairly narrow. Further, exclusion of the first 2.6 years of follow-up did not attenuate the association, arguing against an important role for baseline (sub)acute systemic disease. In addition, excluding men with prevalent CV disease did not materially change the HRs for CV risk. Nevertheless, studies addressing testosterone levels, assessed by mass spectrometry–based techniques, as predictors of CV events in relatively younger and presumably healthier men are of continued interest.

In the present study, low SHBG levels predicted an increased risk of CV events. Ours is the first study demonstrating an association between SHBG and the risk of combined fatal and nonfatal CV events. Two previous studies found an association between low SHBG levels and mortality from CV disease and/or CHD (11,19), whereas other studies found no similar association (12,14,20). Yeap et al. (14) found no association between SHBG and incident stroke/transient ischemic attack. In the present study, the association between SHBG and CV risk was no longer significant after adjustment for testosterone levels, suggesting that the covariation of SHBG with testosterone levels may explain some or all of its association with cardiometabolic risk (18).

In the present study, free testosterone levels were weakly and not significantly associated with CV risk, despite a trend toward an inverse association. Most previous studies of testosterone and CV endpoints did not study free or bioavailable testosterone (8–10,16). Some studies (7,14) found similar associations for free (or bioavailable) and total testosterone, whereas others (11,12) found discordant results. The Massachusetts Male Aging Study (11) reported a direct association between free testosterone and mortality from ischemic heart disease. Thus, data regarding free testosterone and CV events are inconsistent. There are differing opinions regarding the merits of calculated free testosterone values because these estimates show larger variability (34). However, they are mainly influenced (80% of attributable variance) by the variability of the total testosterone assay and less influenced (14%) by the equations used (35). Thus, if sensitive and accurate measurements of testosterone are used, calculated free testosterone should provide an accurate estimate of free testosterone (36). The Vermeulen formula used in the present study has been shown to be in good agreement with equilibrium dialysis measurements (26,27).

Study limitations. The results are based on single measurements of sex steroids and SHBG and may underestimate the true associations. Given the diurnal variation in serum testosterone levels (37), the use of some nonmorning samples may contribute to increased variability and underestimation of serum testosterone levels in the present study. However, the diurnal variation of serum testosterone is less in older men (70 years; 10% lower levels at 4 PM than at 8 AM) (37), and the hour of day was adjusted for in these analyses. Another limitation is that baseline diabetes, hypertension, and CV disease were at least in part self-reported. More important, older adults are often treated with medications that might alter CV risk and/or testosterone levels, which were not examined in this report. In addition, our results are limited to elderly Swedish men. Further, our post-hoc analyses using a dichotomous variable comparing quartile 4 with quartiles 1 to 3 were not adjusted for multiple comparisons. The study also has considerable strengths, including the mass spectrometry–based methodology, the large well-characterized sample, complete follow-up, fatal and nonfatal outcomes, and the documented accuracy of classification in nationwide Swedish registers (28).

Conclusions

Higher serum testosterone levels are associated with a reduced risk of fatal and nonfatal CV events in community-dwelling elderly men.

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