

Low Serum Testosterone and Estradiol Predict Mortality in Elderly Men

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Context: Age-related reduction of serum testosterone may contribute to the signs and symptoms of aging, but previous studies report conflicting evidence about testosterone levels and male mortality. No large prospective cohort study has determined a possible association between serum estradiol and mortality in men.

Objective: The main objective was to examine the association between serum testosterone and estradiol and all-cause mortality in elderly men.

Design, Setting, and Participants: We used specific gas chromatography-mass spectrometry to analyze serum sex steroids at baseline in older men who participated in the prospective population-based MrOS Sweden cohort ($n = 3014$; mean age, 75 yr; range, 69–80 yr).

Main Outcome Measure: All-cause mortality by serum testosterone and estradiol levels.

Results: During a mean follow-up period of 4.5 yr, 383 deaths occurred. In multivariate hazards regression models, low levels (within quartile 1 vs. quartiles 2–4) of both testosterone [hazard ratio (HR), 1.65; 95% confidence interval (CI), 1.29–2.12] and estradiol (HR, 1.54; 95% CI, 1.22–1.95) associated with mortality. A model including both hormones showed that both low testosterone (HR, 1.46; 95% CI, 1.11–1.92) and estradiol (HR, 1.33; 95% CI, 1.02–1.73) predicted mortality. Risk of death nearly doubled (HR, 1.96; 95% CI, 1.46–2.62) in subjects with low levels of both testosterone and estradiol compared with subjects within quartiles 2–4 of both hormones.

Conclusions: Elderly men with low serum testosterone and estradiol have increased risk of mortality, and subjects with low values of both testosterone and estradiol have the highest risk of mortality. (*J Clin Endocrinol Metab* 94: 2482–2488, 2009)

Testosterone gradually declines as men age (1). As testosterone has important physiological effects on, for example, muscle, bone, fat mass, and brain in men, decreased testosterone levels may contribute to the symptoms and signs of aging, *e.g.* decreased muscle mass and strength, impaired physical performance and cognitive function, and lack of energy (1). Men with low serum testosterone are at increased risk of falls, low bone mineral density, and fractures (2–4). Moreover, low serum tes-

tosterone associates with increased fat mass, an adverse metabolic risk profile, and atherosclerosis (1, 5).

Recently, much interest has focused on testosterone supplementation in elderly men (1), as evidenced by a 20-fold increase in testosterone sales in the United States during the 1990s (6). However, few large controlled studies demonstrate the efficacy and long-term safety of testosterone therapy (1, 7). Furthermore, testosterone supplementation has increased, despite relatively

few studies linking androgen deficiency in the elderly to health-related outcomes, including mortality.

Circulating estradiol levels in men are low but measurable, exceeding the levels found in postmenopausal women. Because approximately 80% of circulating estradiol in men derives from androgens (1), serum levels of estradiol and testosterone are significantly associated (2). Studies investigating aromatase or estrogen receptor deficiency in males demonstrated that estradiol has important physiological effects on bone maturation and peak bone mass in younger men (8, 9). The role of estradiol in elderly men remains more unclear, and few studies have explored the relationship between estradiol levels in elderly men and health-related outcomes. However, our earlier study in the Swedish Osteoporotic Fractures in Men (MrOS) cohort showed that low serum estradiol associates with an increased risk of fractures (2).

Among the investigations that assessed the relationship between serum testosterone and mortality in men (6, 10–15), only three were large prospective population-based cohort studies (6, 12, 13). One study showed an association between low serum testosterone and mortality in older men (6), whereas two studies in middle-aged men reported no similar association (12, 13). Currently, no large prospective study has determined a possible association between serum estradiol and mortality in men.

Immunoassay-based techniques provide questionable specificity for measuring sex steroids, especially at low hormone concentrations (16, 17). Indeed, the use of such techniques may contribute to the disparate results of previous studies and also to the paucity of studies on estradiol and clinical outcomes in men. No previous mortality study has used the reference method mass spectrometry to assess sex steroids (18).

This prospective study investigated a possible link between serum testosterone and estradiol levels, assessed by gas chromatography-mass spectrometry (GC-MS), and all-cause mortality in the MrOS Study in Sweden, a large population-based cohort of elderly Swedish men.

Subjects and Methods

Study population

The multicenter MrOS Study includes older men in Sweden, Hong Kong, and the United States. In Sweden, MrOS ($n = 3014$) comprises three subcohorts in three different cities: Malmö ($n = 1,005$), Göteborg ($n = 1,010$), and Uppsala ($n = 999$). Study subjects (men aged 69–80 yr) were selected randomly from national population registers (19). Eligibility for study participation required the ability to walk unassisted, provide self-reported data, and understand and sign an informed consent; 45% of those contacted participated in the study. The MrOS Study in Sweden was approved by the ethics committees at Göteborg, Lund, and Uppsala Universities.

We investigated here the associations between serum sex steroids and mortality in the Swedish MrOS cohort. Levels of SHBG were available for 97% of the entire cohort, and serum samples for sex steroid levels assessed by GC-MS (1 ml required) were available from 99% of the subjects in the Göteborg cohort, 96% in the Malmö cohort, and 68% in the Uppsala cohort.

Assessment of covariates

We used a standardized questionnaire (20) to gather information about smoking habits and physical activity as well as self-reported med-

ical diagnosis (diabetes, cancer, stroke, myocardial infarction, or angina pectoris) by a doctor. This study defined prevalent cardiovascular disease (CVD) as a history of stroke, myocardial infarction, and/or angina pectoris. Physical activity was the subject's estimation of average total daily walking distance, including walking both as a means of exercise and leisure and as a means of outdoor transportation in activities of daily life. We used standard equipment to measure height and weight (2), and calculated body mass index (BMI) as weight (in kilograms)/height (meters)².

Serum analyses

We used a validated GC-MS system (21, 22) to analyze testosterone [detection limit, 0.05 ng/ml; intraassay coefficient of variation (CV), 2.9%; interassay CV, 3.4%] and estradiol (detection limit, 2.00 pg/ml; intraassay CV, 1.5%; interassay CV, 2.7%) in baseline serum samples (2). The blood specimens from the Göteborg cohort and the major part of the Uppsala and Malmö cohorts were obtained between 0800 and 0830 h, but some of the specimens from the Uppsala and Malmö cohorts were obtained between 1230 and 1330 h (approximately 31% of the total number of serum samples included in the present analysis were obtained between 1230 and 1330 h). An HP5973 quadrupole mass spectrometer equipped with a chemical ionization source detected analytes and internal standards. Twenty-six subjects had serum estradiol levels that were below the lower limit of detection. We used immunoradiometric assay (Orion Diagnostics, Espoo, Finland; detection limit, 1.3 nmol/liter; intraassay CV, 3%; interassay CV, 7%) to measure serum SHBG. We calculated free testosterone and free estradiol according to the method described by Vermeulen *et al.* (23) and van den Beld *et al.* (24), taking concentrations of total testosterone, total estradiol, and SHBG into account and assuming a fixed albumin concentration (43 g/liter). All samples were analyzed in one laboratory.

Assessment of mortality

We collected mortality data from the population statistics at Statistics Sweden and recorded follow-up time as the period between baseline visit (in 2004) and date of death or mortality data collection (March 1, 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. The data were collected from this register from the study start until the last update of the register on December 31, 2005 and from evaluation of copies of death certificates for deaths occurring after this date. Based on the information from the register/death certificate, the underlying death cause was determined for each subject. The death causes were then classified as CVD (ICD-10 codes I00 to I99) or other (non-CVD).

Statistical analysis

We used Cox proportional hazards regression to analyze the associations between serum sex steroids and mortality outcomes. Sex steroid levels were examined as quartiles based on the entire population or as dichotomous variables comparing quartile 1 to quartiles 2–4. We also show the effect estimate for a 1 SD increase (z score) of log-transformed sex steroid levels. We adjusted all estimates for age and MrOS site and made further adjustments for BMI (log-transformed), current smoking (yes/no), and physical activity (kilometers walked per day, entered as quartiles because of a nonnormal distribution).

Unadjusted Kaplan-Meier survival curves illustrated the association between testosterone and estradiol status (quartile 1 *vs.* quartiles 2–4) and all-cause mortality, and the log-rank test assessed statistical significance. Spearman rank correlation assessed the univariate association between serum testosterone and estradiol. We performed statistical analyses with SPSS for Windows, version 13.0 (SPSS, Chicago, IL).

TABLE 1. Characteristics of study subjects

Variable	n	
Age (yr)	3014	75.4 (3.2)
BMI (kg/m ²)	3014	26.4 (3.6)
Physical activity (km walked per day)	3014	3.9 (3.1)
Current smoking, % (n)	3014	8.4 (252)
Prevalent disease, % (n)	3014	
Cancer		15.5 (467)
Diabetes		9.5 (286)
Cardiovascular disease		26.5 (798)
Sex steroid levels		
SHBG (nmol/liter)	2925	43.3 (21.9)
Testosterone (ng/ml)	2639	4.50 (1.87)
Estradiol (pg/ml)	2639	20.9 (7.9)
Free testosterone (pg/ml)	2618	80.9 (34.1)
Free estradiol (pg/ml)	2618	0.36 (0.15)

Values are given as mean (sb) unless otherwise indicated.

Results

Table 1 shows the baseline characteristics of the MrOS Sweden cohort (n = 3014). SHBG data (assessed by RIA) and serum sex steroids (assessed by GC-MS) were available for 2925 and 2639 subjects, respectively. The mean follow-up period was 4.5 yr, and the study included 13,527 person-years of follow-up. During the follow-up period, 383 persons (12.7%) died, yielding a mortality rate of 28.3 per 1000 person-years at risk. Because cause of death certificates were missing for 20 of the men who died during the follow-up, death cause was determined for 363 men; 144 (39.7%) of these deaths were due to CVD.

Low physical activity [hazard ratio (HR), 0.88; 95% confidence interval (CI), 0.80–0.96, per quartile increase] and smoking (HR, 1.39; 95% CI, 1.00–1.91; yes *vs.* no) at baseline predicted mortality. There was a nonlinear inverse relation between BMI and mortality [quartile 1 = referent; quartile 2, HR, 0.72 (95% CI, 0.55–0.96); quartile 3, HR, 0.79 (95% CI, 0.60–1.03); and quartile 4, HR, 0.79 (95% CI, 0.60–1.05); quartile 1 *vs.* pooled quartiles 2–4, HR, 0.76 (95% CI, 0.61–0.94)].

Age-adjusted (model 1) proportional hazards regression analyses revealed an association between total and free testosterone levels, total and free estradiol levels, and mortality when sex steroids were entered as quartiles (*P* for trend <0.05) or as continuous variables (Table 2). Further analyses using quartile 1 as reference to allow direct comparison against quartiles 2, 3, and 4 showed that risk of death increased in men within quartile 1 of total and free testosterone levels and total and free estradiol levels compared with quartiles 2, 3, and 4 of each respective hormone, thus revealing a nonlinear association. Results were similar for total and free sex steroid levels. The associations remained after further adjustment for age and BMI (model 2) as well as age, BMI, physical activity, and smoking (model 3). We observed no association between SHBG levels and all-cause mortality (Table 2).

Because subjects within the lowest quartile of estradiol and testosterone levels showed increased risk of mortality, we investigated increased mortality risk in the lowest quartile *vs.* the pooled quartiles 2–4 (Table 2). Compared with subjects within quartiles 2–4, testosterone and estradiol levels within quartile 1 associated with an increased mortality risk of 50 and 60%, re-

spectively (Table 2, model 1, adjusted for age). Further adjustment for age and BMI (model 2) as well as age, BMI, physical activity, and smoking (model 3) showed no major influence on mortality risk at low sex steroid levels (Table 2). Cumulative survival curves (Fig. 1) illustrate that subjects in the lowest quartile of testosterone (Fig. 1A) and estradiol (Fig. 1B) levels had higher all-cause mortality compared with subjects in quartiles 2–4 (log-rank test *P* < 0.001 for both analyses).

Serum levels of total estradiol and testosterone were associated with each other (Spearman rank correlation coefficient 0.54; *P* < 0.001), as were the corresponding free hormone levels (0.62; *P* < 0.001). To study whether low testosterone and estradiol levels independently predict total mortality, we entered both low estradiol (quartile 1 *vs.* quartiles 2–4) and low testosterone (quartile 1 *vs.* quartiles 2–4) in the same hazards regression analyses (Table 2, models 1–3). Low levels of both testosterone and estradiol independently predicted all-cause mortality in these models (Table 2).

To illustrate further the impact of low estradiol and/or testosterone levels, we divided subjects into four groups according to both estradiol and testosterone status: group 1 (referent), with medium/high (= within quartiles 2–4) testosterone and estradiol levels (n = 1667); group 2, with low (= within quartile 1) testosterone but medium/high estradiol levels (n = 312); group 3, with low estradiol but medium/high testosterone levels (n = 307); and group 4, with low levels of both testosterone and estradiol (n = 353). Risk of death approximately doubled [HR, 1.96 (95% CI, 1.46–2.62); model 3, adjusted for age, BMI, physical activity, and smoking] in subjects with low levels of both testosterone and estradiol (group 4) compared with subjects within group 1. In contrast, neither low testosterone [HR, 1.27 (95% CI, 0.87–1.86)] nor low estradiol [HR, 1.23 (95% CI, 0.87–1.74)], with medium/high levels of the other sex hormone, associated with a statistically significant increase in mortality risk. These results were similar in models 1 and 2 (age or age plus BMI adjustment only). Figure 1C shows the survival plots of groups 1–4 (log-rank test *P* < 0.001 for group 4 and nonsignificant for groups 2–3 compared with group 1).

To examine the possible influence of prevalent diseases at baseline on the relationship between sex steroids and mortality, we calculated HRs for mortality after excluding subjects with prevalent cancer, CVD, or diabetes (Table 3). Excluding prevalent diseases showed no major impact on the association between low total or free testosterone levels and all-cause mortality, and there was little impact on the association between low estradiol levels and mortality after exclusion for prevalent CVD or diabetes. Excluding subjects with prevalent cancer attenuated the association between total estradiol and mortality but had less impact on the association between free estradiol and mortality.

To study how follow-up time impacts the association between sex steroids and mortality, we performed analyses that excluded subjects with a follow-up time of 3 yr or less (*i.e.* death within the first 3 yr of follow-up; n = 195 among 383 deaths). However, this exclusion had no major influence on the results [HR (95% CI) for quartile 1 of total testosterone *vs.* quartiles 2–4, 1.72 (1.21–2.43), adjusted for age, BMI, physical activity, and smoking; corresponding analysis for total estradiol, HR, 1.47 (95% CI, 1.06–2.05)].

TABLE 2. HRs of sex steroids in quartiles for mortality

	Model 1	Model 2	Model 3
Total testosterone (ng/ml)			
Q1 (≤ 3.36)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Q2 (3.37–4.38)	0.75 (0.56–1.00)	0.67 (0.50–0.90)	0.71 (0.53–0.96)
Q3 (4.39–5.54)	0.60 (0.44–0.82)	0.53 (0.39–0.73)	0.55 (0.39–0.76)
Q4 (≥ 5.55)	0.66 (0.49–0.89)	0.57 (0.41–0.80)	0.59 (0.42–0.83)
<i>P</i> (trend over quartiles)	0.002	<0.001	<0.001
Risk for 1 sd increase in total testosterone	0.80 (0.74–0.85)	0.78 (0.73–0.83)	0.78 (0.73–0.84)
Free testosterone (pg/ml)			
Q1 (≤ 61)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Q2 (61–79)	0.74 (0.56–0.99)	0.69 (0.52–0.92)	0.70 (0.52–0.94)
Q3 (79–99)	0.62 (0.45–0.84)	0.57 (0.42–0.78)	0.58 (0.42–0.79)
Q4 (≥ 99)	0.56 (0.41–0.78)	0.50 (0.35–0.69)	0.50 (0.36–0.71)
<i>P</i> (trend over quartiles)	<0.001	<0.001	<0.001
Risk for 1 sd increase in free testosterone	0.78 (0.73–0.84)	0.77 (0.72–0.82)	0.77 (0.72–0.83)
Total estradiol (pg/ml)			
Q1 (≤ 16.0)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Q2 (16.0–20.3)	0.57 (0.42–0.77)	0.57 (0.42–0.78)	0.59 (0.43–0.81)
Q3 (20.3–25.3)	0.61 (0.45–0.83)	0.62 (0.46–0.84)	0.65 (0.47–0.88)
Q4 (≥ 25.3)	0.67 (0.50–0.91)	0.68 (0.50–0.91)	0.69 (0.51–0.94)
<i>P</i> (trend over quartiles)	0.018	0.020	0.033
Risk for 1 sd increase in total estradiol	0.77 (0.71–0.83)	0.76 (0.70–0.83)	0.77 (0.71–0.83)
Free estradiol (pg/ml)			
Q1 (≤ 0.27)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Q2 (0.27–0.35)	0.63 (0.47–0.85)	0.64 (0.48–0.87)	0.68 (0.50–0.93)
Q3 (0.35–0.44)	0.55 (0.40–0.75)	0.57 (0.41–0.78)	0.60 (0.43–0.83)
Q4 (≥ 0.44)	0.65 (0.48–0.89)	0.67 (0.49–0.91)	0.69 (0.51–0.95)
<i>P</i> (trend over quartiles)	0.012	0.027	0.043
Risk for 1 sd increase in free estradiol	0.76 (0.70–0.82)	0.76 (0.70–0.82)	0.76 (0.70–0.83)
SHBG (nmol/liter)			
Q1 (≤ 28.8)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Q2 (28.8–38.7)	0.92 (0.68–1.30)	0.88 (0.66–1.19)	0.91 (0.67–1.23)
Q3 (38.7–52.5)	0.95 (0.70–1.28)	0.86 (0.63–1.17)	0.90 (0.66–1.24)
Q4 (≥ 52.5)	1.16 (0.87–1.56)	1.12 (0.81–1.53)	1.14 (0.83–1.58)
<i>P</i> (trend over quartiles)	0.41	0.95	0.80
Risk for 1 sd increase in SHBG	1.10 (0.99–1.22)	1.06 (0.95–1.18)	1.07 (0.96–1.20)
Model including testosterone			
Total testosterone (Q1 vs. Q2–4)	1.50 (1.19–1.89)	1.72 (1.35–2.19)	1.65 (1.29–2.12)
Model including estradiol			
Total estradiol (Q1 vs. Q2–4)	1.60 (1.27–2.02)	1.59 (1.26–2.00)	1.54 (1.22–1.95)
Model including both testosterone and estradiol			
Total testosterone (Q1 vs. Q2–4)	1.30 (1.01–1.67)	1.51 (1.15–1.97)	1.46 (1.11–1.92)
Total estradiol (Q1 vs. Q2–4)	1.45 (1.12–1.86)	1.36 (1.05–1.75)	1.33 (1.02–1.73)

Data are expressed as HR (95% CI). Model 1, adjusted for age, MrOS site; model 2, adjusted for age, MrOS site, and BMI; model 3, adjusted for age, MrOS site, BMI, physical activity, and current smoking. Q, Quartile.

To analyze further the impact of BMI on the relation between sex hormones and mortality, we calculated the age-adjusted HR of low testosterone or low estradiol (quartile 1 vs. pooled quartiles 2–4) for mortality within each quartile of BMI. Within BMI quartile 1, the HR (95% CI) was 1.79 (1.12–2.87) for low testosterone and 1.62 (1.09–2.41) for low estradiol; the corresponding HRs within BMI quartile 2 were 1.89 (1.14–3.11) and 1.61 (1.00–2.60); within BMI quartile 3, 1.34 (0.82–2.20) and 1.40 (0.83–2.35); and within BMI quartile 4, 1.52 (0.97–2.39) and 1.68 (1.04–2.71).

To study whether there was an increased incidence of cardiovascular deaths, we analyzed the risk of CVD and non-CVD death at low hormone levels. The risk of death from noncardiovascular causes [age-adjusted HR (95% CI) for quartile 1 of total testosterone vs. quartiles 2–4, 1.75 (1.30–2.37); corresponding analysis for total estradiol, 2.00 (1.49–2.69)] was increased at

low hormone levels. In contrast, although there was a tendency, the risk of death from cardiovascular causes was not significantly increased [age-adjusted HR (95% CI) for quartile 1 of total testosterone vs. quartiles 2–4 was 1.21 (0.81–1.79); corresponding analysis for total estradiol, 1.22 (0.82–1.81)].

Discussion

This prospective study investigated a possible link between serum testosterone and estradiol levels, assessed by GC-MS, and mortality in a large population-based cohort of elderly men. Our results show that risk of death increased for elderly men in the lowest quartile of both testosterone and estradiol levels. Testosterone and estradiol predicted death independently of each other,

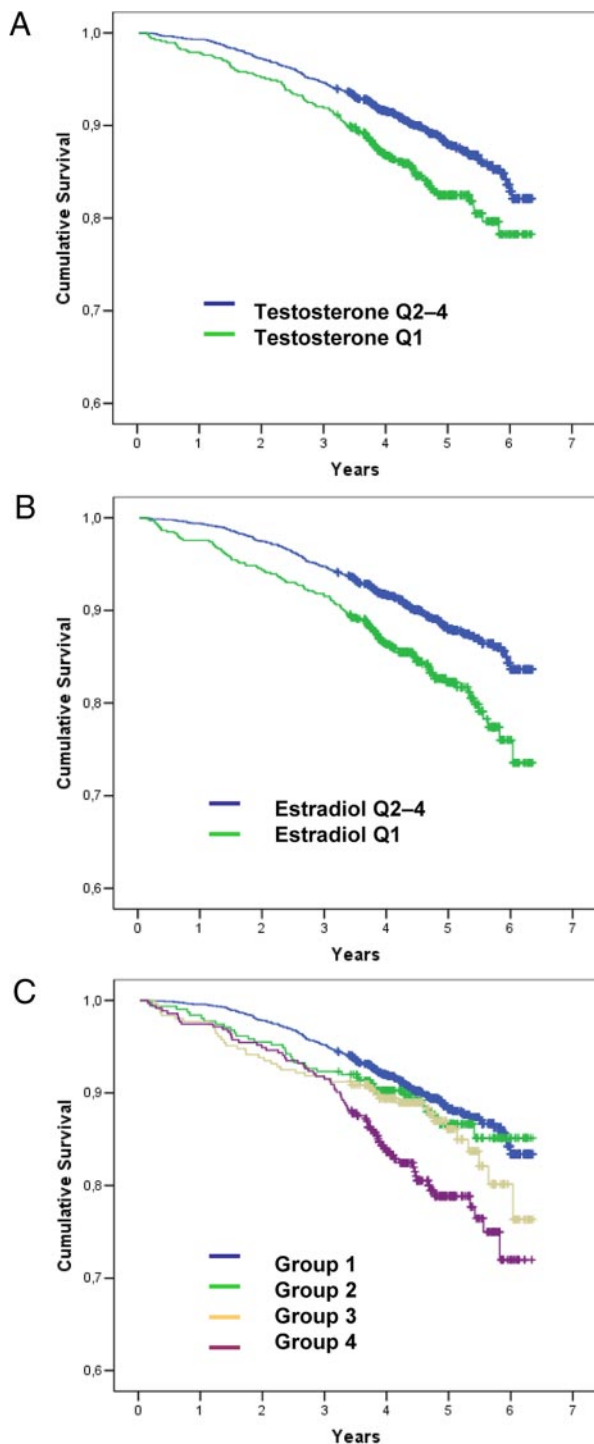


FIG. 1. Unadjusted Kaplan-Meier survival curves according to serum sex steroid levels. A, Cumulative survival of subjects within the lowest Q of serum total testosterone compared with Q2–4. B, Cumulative survival of subjects within the lowest Q of serum total estradiol compared with Q2–4. C, Cumulative survival of subjects within the lowest quartile of both serum total testosterone and estradiol. Group 1, Medium/high testosterone and medium/high estradiol; group 2, low testosterone and medium/high estradiol; group 3, low estradiol and medium/high testosterone; group 4, low testosterone and low estradiol. Low estradiol, Subjects within the lowest Q of estradiol (≤ 16 pg/ml); medium/high estradiol, subjects within Q2–4 of estradiol (> 16 pg/ml); low testosterone, subjects within the lowest Q of testosterone (≤ 3.36 ng/ml); medium/high testosterone, subjects within Q2–4 of testosterone (> 3.36 ng/ml). Q, Quartile.

TABLE 3. HRs of low levels of sex steroids (quartile 1 vs. quartiles 2–4) for mortality, excluding subjects with prevalent disease

	Model 3
HR of low total testosterone for mortality (Q1 vs. Q2–4)	<i>1.65 (1.29–2.12)</i>
Excluding prevalent cancer	1.57 (1.17–2.10)
Excluding prevalent CVD	1.79 (1.30–2.46)
Excluding prevalent diabetes	1.69 (1.28–2.22)
HR of low free testosterone for mortality (Q1 vs. Q2–4)	<i>1.65 (1.30–2.11)</i>
Excluding prevalent cancer	1.45 (1.08–1.93)
Excluding prevalent CVD	1.83 (1.34–2.51)
Excluding prevalent diabetes	1.68 (1.28–2.19)
HR of low total estradiol for mortality (Q1 vs. Q2–4)	<i>1.54 (1.22–1.95)</i>
Excluding prevalent cancer	1.27 (0.96–1.69)
Excluding prevalent CVD	1.58 (1.17–2.13)
Excluding prevalent diabetes	1.60 (1.24–2.06)
HR of low free estradiol for mortality (Q1 vs. Q2–4)	<i>1.46 (1.15–1.86)</i>
Excluding prevalent cancer	1.34 (1.01–1.78)
Excluding prevalent CVD	1.35 (0.99–1.85)
Excluding prevalent diabetes	1.50 (1.16–1.95)

Data are expressed as HR (95% CI). Model 3, Adjusted for age, MRos site, BMI, physical activity, and current smoking. Q, Quartile. *Italics* indicate reference data from Table 2.

and risk of death nearly doubled (96% increase) in subjects with both low testosterone and low estradiol compared with subjects within quartiles 2–4 of both hormones.

To our knowledge, ours is the first study that shows estradiol as a predictor of mortality in elderly men. Furthermore, the present study is the first large study to report on the association between estradiol and testosterone, assessed by the reference method GC-MS (16, 17), and mortality (18). The absence of previous studies demonstrating an association between low estradiol levels and mortality may result partly from the use of immunoassay-based techniques, which may provide questionable specificity at low estradiol levels (17). Moreover, such technical restraints may explain the paucity of studies on serum estradiol levels and other health-related outcomes in men. However, our recent study on this cohort reported that older men with low serum estradiol, assessed by GC-MS, have increased risk of fractures (2). Therefore, it is reasonable to believe that more sensitive and reliable techniques, such as GC-MS, will help unravel important physiological effects of estradiol in men.

Three previous prospective population-based cohort studies investigated the relationship between serum testosterone and total mortality (6, 12, 13). Although two studies observed no association between testosterone and survival (12, 13), Laughlin *et al.* (6) showed that low testosterone associated with increased mortality in men in the Rancho Bernardo Study. The two negative studies investigated middle-aged men (mean age, 52 and 55 yr, respectively), whereas Laughlin *et al.* (6) studied elderly men with a mean age comparable to that of the present study, possibly accounting for the different results. In the study by Laughlin *et al.* (6), 68% of the cohort (mean age, 71 yr; range, 50–91) died during an average 11.8 yr of follow-up, yielding a mortality rate of 57.5 per

1000 person-years at risk. In the present study, 13% of subjects (mean age, 75 yr; range, 69–80 yr) died during an average 4.5 yr of follow-up, yielding a mortality rate of 28.5 per 1000 person-years at risk. Thus, our studied subjects are a few years older at baseline and have a smaller age span, a shorter follow-up time, and a lower mortality rate compared with the Laughlin study. Furthermore, whereas Laughlin *et al.* (6) used an immunoassay-based method to assess testosterone, we used GC-MS (16, 17). Despite these differences, both studies found that testosterone levels within the lowest quartile associated with increased risk of all-cause death (38% for Laughlin *et al.* vs. 65% in the present study). Nested case-control, retrospective, and smaller studies also support a link between low testosterone and all-cause mortality in elderly men (10, 11, 14, 15). We observed no relationship between SHBG levels and all-cause mortality, supporting an earlier prospective study (25).

Interestingly, low estradiol and low testosterone predicted death independently of each other, and subjects with low levels of both testosterone and estradiol showed the highest risk of mortality in the present study. These results suggest that both hormones contribute additively to risk of death and that both low testosterone and low estradiol may serve as markers of mortality risk in elderly men.

We propose two major hypotheses regarding the association between low sex steroid levels and mortality: 1) low sex steroid levels cause or worsen disease and thereby cause death; or 2) low sex steroid levels are a result of disease and therefore associate with death. The second hypothesis, *i.e.* low testosterone/estradiol is an epiphenomenon of preexisting diseases, is supported by evidence that both acute and chronic illnesses reduce testosterone production (1, 26). In acute illness, testosterone is often profoundly depressed through mechanisms that act both directly at the testicular level and indirectly through gonadotropin suppression (1, 26). Furthermore, hypogonadism due to primary testicular failure (*e.g.* cytokines acting directly upon the testes) accompanies many chronic diseases such as renal disease, alcoholic liver disease, and rheumatic diseases (1, 26). Therefore, low serum sex steroids might represent a general marker of poor health and thereby associate with increased mortality risk. In our study, the association between testosterone/estradiol and mortality remained significant even after we excluded deaths that occurred during the first 3 yr of follow-up, thus arguing against a substantial role of prevalent diseases for our observations. Furthermore, excluding subjects with prevalent diseases (cancer, CVD, or diabetes) had no major impact on the association between low sex steroid levels and mortality, although excluding subjects with prevalent cancer slightly attenuated the association between total estradiol and mortality. The hypothesis that low sex steroid levels worsen disease and thereby may cause death is supported by the fact that testosterone has important physiological effects on, for example, muscle, bone, fat mass, and brain in men (1). Thus, low testosterone levels may contribute to frailty that affects the individual's capability to recover after any disease event and thereby affects survival. Furthermore, men with low serum testosterone are at increased risk of falls, low bone mineral density, and fractures (2–4). In addition, a pathogenic role of low testosterone in the development of the metabolic syndrome has been suggested (18, 27). Thus, there are several putative mechanisms by which low testosterone could contribute to increased mortality, but additional studies are required.

Because androgen deprivation therapy (chemical or surgical) during treatment of prostate cancer lowers serum testosterone levels, such treatment may affect the association between low testosterone levels and mortality. However, our result that excluding subjects with self-reported cancer at baseline did not significantly influence the association between low testosterone and mortality does not support androgen deprivation therapy as a pivotal factor for the results of this study.

Although there was a tendency, we did not find a statistically significant association between low testosterone (HR, 1.21) or estradiol levels (HR, 1.22) and CVD mortality risk in this cohort of older men. Several previous prospective studies show no significant association between testosterone levels and CVD mortality in men (12, 13, 28–30), whereas an association between testosterone levels and CVD death was found in the prospective cohort study of Laughlin *et al.* (6) (HR, 1.36) as well as in a recent nested case-control study of Khaw *et al.* (15). To our knowledge, there are only two previous, smaller prospective studies on estradiol and CVD death in men, and these studies showed no significant association (28, 31).

In this study, the HRs for mortality were similar for total and free hormone levels. Because obesity is associated with low SHBG (30), somewhat more diverging results for free and total hormone levels could be expected. On the other hand, this population of men is only mildly overweight (average BMI, 26.4 kg/m²). In accordance with the present data, the HRs of free and total testosterone/estradiol levels for incident fractures in this cohort were also similar (2).

We found that subjects with low BMI (within quartile 1) had an increased risk of death compared with subjects within quartiles 2–4, in accordance with most studies reporting either no relation, an inverse or a U-shaped relation between BMI and all-cause mortality in the elderly (32). The relation between low testosterone and estradiol and mortality was rather consistent across BMI quartiles, but with a slight tendency to be U-shaped with the lowest HR of both hormones for mortality within BMI quartile 3, indicating that the sex hormone status is somewhat more predictive of survival in subjects with low or high BMI. Importantly, BMI does not distinguish fat mass from lean mass, and BMI in the lower range is a less valid indicator of body fatness in the elderly in which low BMI rather may indicate low lean mass (33).

Our study has limitations. The fact that only a total of 45% of the subjects who were contacted participated in the study may restrict the generalizability of our findings. The results are based on single measurements of sex steroids and may underestimate the true associations between the markers we studied and the risk of death. Although most of the blood specimens in this study were obtained between 0800 and 0830 h, some samples were obtained between 1230 and 1330 h, and given the well-documented diurnal variation in serum testosterone levels (34), this may contribute to increased variability and underestimation of serum testosterone levels in the present study. However, a recent study showed that the diurnal variation of serum testosterone is less in older men (70 yr), with 10% lower levels at 1600 h than at 0800 h, compared with a 20–25% difference in men 30–40 yr old (34). A population-based study such as ours could imply inclusion of subjects treated with compounds that affect mortality risk and/or sex steroid levels, thus affecting interpretation of our results. In addition, our results are limited to elderly Caucasian men. A large

number of analyses have been performed in the present study, and problems associated with multiple testing may complicate the interpretation of results. However, our main hypothesis, *i.e.* that low serum testosterone and estradiol predict all-cause mortality, was directly assessed by only a small number of analyses, and the other analyses should be considered as exploratory subanalyses.

In conclusion, low serum testosterone and estradiol, assessed by specific GC-MS, associate with risk of death in older Swedish men, and subjects with low values of both testosterone and estradiol have the highest risk of mortality. Thus, both low testosterone and low estradiol may serve as markers of mortality risk in elderly men.

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