Endogenous Testosterone and Mortality in Men: A Systematic Review and Meta-Analysis

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Context: Low testosterone levels have been associated with outcomes that reduce survival in men.

Objective: Our objective was to perform a systematic review and meta-analysis of published studies to evaluate the association between endogenous testosterone and mortality.

Data Sources: Data sources included MEDLINE (1966 to December 2010), EMBASE (1988 to December 2010), and reference lists.

Study Selection: Eligible studies were published English-language observational studies of men that reported the association between endogenous testosterone and all-cause or cardiovascular disease (CVD) mortality. A two-stage process was used for study selection. 1) Working independently and in duplicate, reviewers screened a subset (10%) of abstracts. Results indicated 96% agreement, and thereafter, abstract screening was conducted in singlicate. 2) All full-text publications were reviewed independently and in duplicate for eligibility.

Data Extraction: Reviewers working independently and in duplicate determined methodological quality of studies and extracted descriptive, quality, and outcome data.

Data Synthesis: Of 820 studies identified, 21 were included in the systematic review, and 12 were eligible for meta-analysis [n = 11 studies of all-cause mortality (16,184 subjects); n = 7 studies of CVD mortality (11,831 subjects)]. Subject mean age and testosterone level were 61 yr and 487 ng/dl, respectively, and mean follow-up time was 9.7 yr. Between-study heterogeneity was observed among studies of all-cause ($P < .001$) and CVD mortality ($P = 0.06$), limiting the ability to provide valid summary estimates. Heterogeneity in all-cause mortality (higher relative risks) was observed in studies that included older subjects ($P = 0.020$), reported lower testosterone levels ($P = 0.018$), followed subjects for a shorter time period ($P = 0.010$), and sampled blood throughout the day ($P = 0.030$).

Conclusion: Low endogenous testosterone levels are associated with increased risk of all-cause and CVD death in community-based studies of men, but considerable between-study heterogeneity, which was related to study and subject characteristics, suggests that effects are driven by differences between cohorts (*e.g.* in underlying health status). **(*J Clin Endocrinol Metab* 96: 3007–3019, 2011)**

Serum testosterone levels exhibit a gradual decline as men age, with longitudinal studies showing declines between 0.4 and 2.6% per year (1, 2). At the same time, SHBG rises, resulting in a greater decline (≥2% per year) in free testosterone (2). The prevalence of late-onset hypogonadism (defined according to recent clinical guidelines (3) as presence of clinical symptoms and low testosterone levels) is less than 10% (4–6).

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Abbreviations: BMI, Body mass index; CI, confidence interval; CVD, cardiovascular disease; RR, relative risk.

In observational studies, low serum testosterone and late-onset hypogonadism have been associated with abdominal obesity (4, 5), cardiovascular risk factors and the metabolic syndrome (6–11), type 2 diabetes mellitus (12–14), increased inflammatory biomarkers (15, 16), and dyslipidemia (17–19) among other outcomes (20) that may increase risk of premature death. In recent years, several observational studies have examined the association between endogenous testosterone levels and mortality. Many, but not all, of these studies have shown that low testosterone levels are associated with increased overall and cardiovascular disease (CVD) mortality. Pooling of data from these studies in a systematic review and meta-analysis may provide clarity to the conflicting data or identify study or subject characteristics associated with variation in results among studies.

The primary objective of this research is to assess the association between endogenous testosterone levels and all-cause and CVD mortality from observational studies conducted in men. Secondarily, it will assess whether important clinical [*e.g.* baseline testosterone level, smoking status, and body mass index (BMI)], demographic (*e.g.* age), or study-related (*e.g.* length of follow-up and type of testosterone assay) factors influence or modify study results.

Materials and Methods

Eligibility criteria

Eligible studies were defined in a protocol as fully published (English language) observational studies that assessed the association between endogenous testosterone level and all-cause or CVD mortality in men. To be included in this analysis, the studies had to have a measure of association available or the possibility to calculate this from data presented in the article. We excluded studies of exogenous administration of testosterone as well as studies not reporting at least one of the outcomes of interest. In the event of overlapping reports using the same population, the one with the largest number of subjects and the longest follow-up period was used. No attempt was made to contact authors for additional information.

Search strategy

To identify eligible studies, we conducted a systematic search of the literature using the electronic databases MEDLINE (1966 to December 2010) and EMBASE (1988 to December 2010) and reviewed reference lists from included studies. Two search strategies were employed: one was more specific, and one was more sensitive. Results were combined to produce a comprehensive list of potential eligible references. The search logic for both searches can be found in Supplemental Tables 1 and 2 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Working independently and in duplicate, reviewers (J.M.D. and E.A.S.) screened a subset (10%) of abstracts. Results indicated 96% agreement, and thereafter, abstract screening was conducted in singlicate. All full-text publications were reviewed independently and in duplicate for eligibility. In cases in which disagreement between reviewers existed, the research team lead (A.B.A.) reviewed the publication and determined eligibility.

Data extraction for systematic review

Using a standardized data extraction form and working in duplicate, the following data were abstracted from each publication: study characteristics (author, publication year, study design, number of subjects, length of follow-up, testosterone collection/assay procedures, adjustment for covariates, *etc.*), sample characteristics [mean age, BMI, current smoking status, testosterone level, and number of deaths (overall and CVD)], and study results [measure of association as assessed by relative risk (RR), odds ratio, or hazard ratio of death per SD or in quantiles and 95% confidence intervals (CI)].

Data extraction for quality of study reporting

Independent critical appraisal of included studies was conducted by the two reviewers using as a guide statements such as the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) (21) and the Newcastle-Ottawa Quality Assessment Scale for cohort studies. Specifically, the following parameters were considered: representativeness (judged by the response rate to the baseline survey), adequacy of the follow-up period (>1 yr), adequacy of follow-up for outcome of interest (*i.e.* low lost-to-follow-up rate), and ascertainment of exposure [including morning blood sampling (yes/no) and type of testosterone assay (platform-based immunoassay/gas chromatography tandem mass spectrometry, liquid chromatography tandem mass spectrometry, or RIA with extraction), exclusion of subjects on treatment (*e.g.* testosterone or other hormones or antiandrogens) that would profoundly affect testosterone levels (yes/no), and comparability of cohorts on the basis of the design or analysis (assessed by the number and type of covariate adjustment)]. When reviewers' conclusions over the validity of a study differed, the study was reviewed jointly with research team lead (A.B.A.).

Data extraction for relative risks

We extracted the RR, odds ratio, or hazard ratio, and associated 95% CI with the largest number of adjustment variables from each study, excluding other hormones (*e.g.* SHBG and estradiol).

Statistical analysis

Although most of the studies identified for meta-analysis (22–29) reported measures of association in terms of 1-SD changes in testosterone, other studies reported measures of association in terms of equal (*i.e.* quantile comparisons) or unequal size groups (*i.e.* two or three groups defined by testosterone cut-points). We converted the reported RR estimates onto a standard scale of effect, comparing the lowest third with the highest third of the testosterone distribution. This is similar to providing an estimate per 2.18 SD units of testosterone, where 2.18 is the difference in the means of the top and bottom third of the standard normal distribution (30). Measures of association were placed on the same scale under the assumptions that testosterone is log normally distributed and that the association with mortality risk is log-linear. These assumptions were verified in our Massachu-

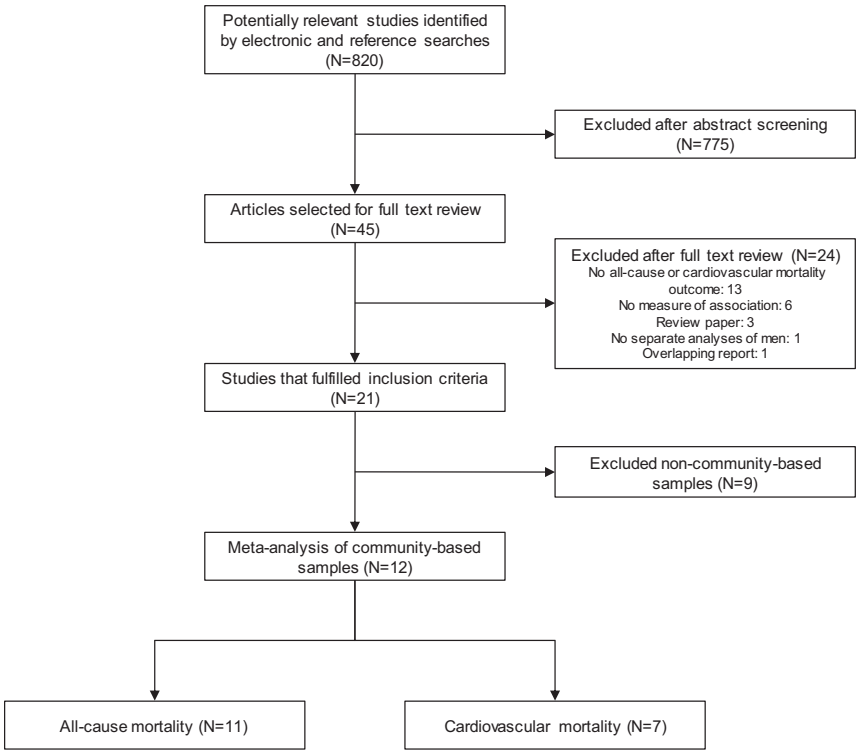

FIG. 1. Results of the systematic search.

setts Male Aging Study (MMAS) database (22). For studies reporting measures of association in terms of 1 SD (22–29), 2.18 was used as the scaling factor for the natural logarithm of the RR. For studies reporting four (31) and 10 (32) equal groups, we used scaling factors of 2.18/2.54 and 2.18/3.53, respectively, *i.e.* the difference in means between the top and bottom tertile in each case under the assumption of log normality for testosterone. In the case of studies reporting data on two unequal groups (33, 34), we used study-specific scaling factors that were calculated as $2.18/x$ where x is the difference in mean testosterone (in SD units) between the unequal groups.

Summary or pooled RR estimates and corresponding 95% CI were derived by using the DerSimonian-Laird random-effects model, which incorporates between-study variability (35). Statistical heterogeneity between studies was evaluated with the I^2 statistic (36). Where statistical pooling was judged as not possible, the findings were presented in narrative/tabular format. Where statistical pooling was possible, we used the natural logarithm of the reexpressed RR estimate with its SE for the meta-analysis. Prespecified sensitivity analyses were performed with metaregression models of the natural logarithm of the RR as the dependent variable and subject (age, BMI, smoking, and testosterone level) or study characteristics (length of follow-up, number of covariates adjusted, geographic location, type of testosterone assay, morning blood sampling, exclusion of subjects on treatments that profoundly affect testosterone, and response rate) as the independent variable. Variables (all dichotomized) were examined for their contribution to heterogeneity one at a time, with no multivariate modeling due to the relatively small number of studies included. Age, BMI, smoking, testosterone level, length of follow-up, number of covariates adjusted, and response rate were dichotomized at the median observed in the studies. The remaining variables were defined as follows: type of testosterone assay (liquid chromatography-tandem mass spectrometry, gas chromatography-tandem mass spectrometry, or RIA with extraction *vs.* RIA without extraction or platform-based immunoassay), geographic location (United States *vs.* non-United States), morning blood sampling (yes/no), exclusion of subjects on treatment (*e.g.* testosterone or other hormones or antiandrogens) that would profoundly affect testosterone levels (yes/no). Publication bias was assessed with funnel plots, but these provided limited information in the context of the meta-analysis described below, due to the small number of studies included. Statistical analyses were performed using R version 2.12.1 (37). P values <0.05 were considered statistically significant. All statistical tests were two sided.

Results

Search results

Results of the systematic search are shown in Fig. 1. A total of 820 studies were identified and 775 were excluded after abstract screening. Of the 45 articles selected for full-text review, 24 were excluded after full-text review, leaving 21 studies that were included in the systematic review. These studies are described in Table 1 (community-based) and Table 2 (non-community-based). Study methods and populations for the nine (9) non-community-based (generally clinic-based) studies varied widely (described further below), thus prohibiting statistical pooling. The remaining community-based studies were subject to meta-analysis (n = 11 for all-cause and n = 7 for CVD mortality). The data from the two Rancho-Bernardo studies (23, 31) were described separately because the first (23) was included in the meta-analysis of CVD mortality, and the second (31) was included in the meta-analysis of all-cause mortality. The latter was excluded from the meta-analysis of CVD mortality due to overlap with the former. The data from the Menke publication (32) were presented stratified by time (baseline to 9 yr and 9–18 yr). As a result, only the baseline to 9 yr data were included in the meta-analysis because the two cannot be considered independent. Selected data used in the meta-analysis are presented in Table 1.

Quality of study reports

Results of the quality review of community-based studies are presented in Table 3. Overall, these studies were of high quality, with notable exceptions. First, the reporting of response rates to the baseline surveys was incomplete (four of the 12 studies did not report the response rate or sufficient data to calculate it), and response rates ranged from 25–89%. All studies followed subjects for an adequate amount

TABLE 1. Characteristics of community-based studies included in the all-cause or CVD mortality meta-analysis

Primary author, year (Ref.)	Subjects	Mean follow-up (yr)	Mean age (yr)	Mean BMI (kg/m^2)
Araujo, 2007 (22)[a,b]	1686 men from the MMAS (Boston, MA)	15.3	55	27.3
Barrett-Connor, 1988 (23)[b]	872 men (Rancho Bernardo, CA)	12	63	NR
Haring, 2010 (33)[a,b]	1954 men from SHIP (West Pomerania, Germany)	7.2	58.7	27.4
Khaw, 2007 (24)[a,b]	2314 men from EPIC-Norfolk (United Kingdom)	7	67.3	26.7
Laughlin, 2008 (31)[a]	794 men (Rancho Bernardo, CA)	11.8	71.2	25.7
Lehtonen, 2008 (25)[a]	187 home-dwelling male residents (Turku, Southwest Finland)	10	71.5	25.6
Menke, 2010 (32)[a,b]	1114 men from NHANES III (U.S.) (baseline - year 9)	8	40	NR
Shores, 2006 (34)[a]	858 male veterans (U.S.)	4.3	61.4	29.1
Smith, 2005 (26)[a,b]	2323 men from the Caerphilly study (United Kingdom)	16.5	52.1	NR
Szulc, 2009 (27)[a]	782 men from the MINOS study (France)	10	65.4	28
Tivesten, 2009 (28)[a]	2639 elderly men from MrOS (Sweden)	4.5	75.4	26.4
Vikan, 2009 (29)[a,b]	1568 men from the 4th Tromso Study (Norway)	11.2	59.6	NR

International Classification of Diseases, Revision 8 (ICD-8) (400–438) includes hypertensive disease (400–404), ischemic heart disease (410–414), other forms of heart disease (420–429), and cerebrovascular disease (430–438). ICD-9 (390–458, 250, 795, 798) includes acute rheumatic fever (390–392), chronic rheumatic heart disease (393–398), hypertensive diseases (401–405), ischemic heart disease (410–414), diseases of pulmonary circulation (415–417), other forms of heart disease (420–429), cerebrovascular disease and diseases of arteries (430–438), arterioles and capillaries (440–448), diseases of veins and lymphatics, and other diseases of circulatory system (451–459), diabetes mellitus (250), nonspecific abnormal histological and immunological findings (795), and sudden death, cause unknown (798). ICD-10 (I00-I99, R96, R98, R99) includes acute rheumatic fever (I00-I02), chronic rheumatic heart diseases (I05-I09), hypertensive diseases (I10-I15), ischemic heart disease (I20-I25), pulmonary heart disease and diseases of pulmonary circulation (I26-I28), other forms of heart disease (I30-I52), cerebrovascular diseases (I60-I69), diseases of arteries, arterioles, and capillaries (I70-I79), diseases of

of time, and loss to follow-up was very low. Seven of the 12 studies obtained morning blood samples, and seven of 12 studies used high-quality testosterone assays. Five studies excluded subjects on treatments that profoundly affect testosterone levels, and statistical adjustments were judged to be adequate in all studies.

Meta-analysis

Described in Table 1 are the characteristics of the 12 community-based studies that were included in both the systematic review and meta-analysis. Mean age and testosterone level of the subjects in the 12 studies subject to meta-analysis were 61 yr and 487 ng/dl, and they were followed for 9.7 yr on average. Mean BMI in the cohorts was 26.9 kg/m^2, and the average prevalence of current smoking was 28%. The 11 studies on all-cause mortality that were included in the meta-analysis included a total of 16,184 subjects. The seven studies on CVD mortality that were included in the meta-analysis included a total of 11,831 subjects.

TABLE 1. Continued

Current smokers (%)	Mortality ascertainment	CVD ascertainment	No. deaths, all-cause	No. deaths, CVD	Mean total T (ng/dl)
24.0	National Death Index	ICD-9 (410–414) and ICD-10 (I20–I25)	395	101	517.0
NR	Death certificates coded by certified nosologist	ICD-8 (400–438)	NR	114	536.1
34.3	Death certificates coded by certified nosologist and two independent internists	ICD-10 (I10–I79)	193	68	458.2
10.7	Death certificates from the National Statistics United Kingdom, coded by trained nosologists	ICD-9 (400–448) and ICD-10 (I10-I79)	825	369	472.6
11.0	Death certificates coded by certified nosologist	ICD-9 (401–448)	538	264	300.0
NR	National Death Register kept by Statistics Finland	National Death Register kept by Statistics Finland; causes were categorized as cardiovascular or other	68	27	634.0
NR	National Death Index	ICD-9 (390–434, 436–459) and ICD-10 (I00-I99)	103	42	514.0
NR	National VA death registry, Beneficiary Identification and Records Locator System–Death File, and the regional VA database	NR	208	NR	422.6
55.1	Records at the National Health Service Central Registry were flagged so notification of death was automatic; copy of death certificate was received	ICD-9 (410–414)	482	192	659.3
56.2	Dates of death were obtained through the SSBM insurance rolls	NR	182	NR	511.2
8.4	Swedish Cause of Death Register, held by the National Board of Health and Welfare in Sweden	ICD-10 (I00–I99)	383	144	450.0
33.1	Data from the Norwegian Registry of Vital Statistics; all possible events were reviewed by examining medical records, death certificates, and autopsy reports	ICD-9 (390–458, 250, 795, 798) and ICD-10 (I00-I99, R96, R98, R99)	395	130	383.3

veins, lymphatic vessels, and lymph nodes, not elsewhere classified (I80-I89), other and unspecified disorders of the circulatory system (I95-I99), other sudden death, cause unknown (R96), unattended death (R98), and other ill-defined and unspecified causes of mortality (R99). To convert testosterone in nanograms per deciliter to nanomoles per liter, multiply by 0.0347. EPIC-Norfolk, European Prospective Investigation into Cancer in Norfolk; MMAS, Massachusetts Male Aging Study; MrOS, Osteoporotic Fractures in Men; NHANES III, Third National Health and Nutritional Examination Survey; NR, not reported; SHIP, Study of Health in Pomerania; SSMB, Société de Secours Miniére de Bourgogne; T, testosterone; VA, Veterans Affairs.

[a] Included in the meta-analysis of all-cause mortality.

[b] Included in the meta-analysis of CVD mortality.

Between-study heterogeneity was observed among studies of all-cause ($I^2 = 77.9\%$, $P < 0.001$) and CVD mortality ($I^2 = 49.6\%$, $P = 0.06$), limiting the ability to provide valid summary estimates (see Fig. 2). Examination of funnel plots provided no indication of publication bias (data not shown).

Subject and study characteristics were tested in relation to heterogeneity in RR for all-cause mortality (Table 4). Several factors were unrelated to heterogeneity, including BMI, prevalence of current smoking, number of covariates adjusted, geographic location, type of testosterone assay, study response rate, and whether investigators excluded subjects for treatments that profoundly affect testosterone levels. In contrast, several factors significantly contributed to heterogeneity in the RR across studies. These included the mean age of the population ($P = 0.020$), baseline total

TABLE 2. Characteristics of non-community-based studies not included in the meta-analysis

Primary author, year (Ref.)	Subjects	Mean follow-up	Mean Age (yr)	Mortality ascertainment
Angstwurm, 2005 (44)	208 ICU patients with severe infection (Germany)	28 d	59	In-hospital survival time
Carrero, 2009 (45)	126 hemodialysis patients (Sweden)	41 months	63 (median)	Medical chart review
Corona, 2010 (46)	1475 patients attending an andrological unit for ED (Italy)	4.3 yr	52.9	Records from the City of Florence Registry Office
Guder, 2009 (47)	191 males with systolic or nonsystolic HF enrolled in the Wurzburg Heart Failure Registry (Germany)	~2.4 yr (median)	64.4	Patient status (dead or alive) was ascertained by contacting the patient's general practitioner or consulting hospital discharge letters
Haffner, 1996 (48)	123 older-onset diabetic men enrolled in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (U.S.)	4 yr	67.4	NR
Jankowska, 2006 (49)	208 chronic HF patients and 366 healthy controls (Poland)	~2.5 yr	63 (median)	NR
Jankowska, 2009 (50)	501 men with chronic HF and reduced LVEF (Poland)	~2.2 yr	58	Obtained directly from patients or their relatives, from the CHF clinic database or from the hospital system
Militaru, 2010 (51)	126 male patients admitted to cardiology center with acute MI (Romania)	30 d	62	Hospital charts or information provided by the patient's family
Shores, 2004 (52)	44 men in the Geriatric Evaluation and Management Unit at the VA Puget Sound Health Care System (U.S.)	6 months	75.4	VA clinical records

International Classification of Diseases, Revision 9 (ICD-9) (390–458, 250, 795, 798) include acute rheumatic fever (390–392), chronic rheumatic heart disease (393–398), hypertensive diseases (401–405), ischemic heart disease (410–414), diseases of pulmonary circulation (415–417), other forms of heart disease (420–429), cerebrovascular disease and diseases of arteries (430–438), arterioles and capillaries (440–448), diseases of veins and lymphatics, and other diseases of circulatory system (451–459), diabetes mellitus (250), nonspecific abnormal histological and immunological findings (795), and sudden death, cause unknown (798). To convert testosterone in nanograms per deciliter to nanomoles per liter, multiply by 0.0347. CHD, coronary heart disease; ED, erectile dysfunction; HF, heart failure; HR, hazard ratio; ICU, intensive care unit; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NR, not reported; OR, odds ratio; Q, quartile; VA, Veterans Affairs.

TABLE 2. Continued

CVD ascertainment	No. deaths, all-cause	No. deaths, CVD	Mean total T (ng/dl)	All-cause mortality results	CVD mortality results
NR	67	NR	770	No significant difference in survival between T quartiles	NR
Medical chart review; CVD death defined as death resulting from CHD, sudden death, stroke, or complicated peripheral vascular disease	65	38	287 (median)	Total T <233 ng/dl; HR = 2.21 (1.28–3.82); $P = 0.0004$	Total T <233 ng/dl; HR = 2.79 (1.21–6.48); $P = 0.01$
ICD-9 (410–414, 420–429, 430–434, 436–438 440,798–799)	88	15	475	Low total T not associated with overall death (estimate NR)	Total T < 230 ng/dl; HR = 7.1 (1.8–28.6); $P < 0.001$
NR	53	NR	359 (median)	Total T (per 100 ng/dl); HR = 0.88 (0.73–1.07); $P = 0.205$	NR
ICD-9 (410–414)	NR	41	453	NR	Total T per SD; OR = 1.03 (0.66–1.62); $P = 0.90$
Obtained directly from patients or their relatives, from the CHF clinic database or from the hospital system	75 (all CVD)	75	360–430	NR	Total T per 100 ng/dl; HR = 0.84 (0.72–0.97); $P = 0.02$
NR	171	NR	354 (median)	Total T per 218 ng/dl; HR = 0.79 (0.68–0.91); $P = 0.002$	NR
Hospital charts, death certificates	16	12	410	Total T OR: Q1, 1.00 (ref); Q2, 0.72 (0.51–1.00); Q3, 0.61 (0.43–0.81); Q4, 0.56 (0.40–0.83); P trend <0.01	NR
NR	10	NR	293	Total T <300 ng/d; HR = 27.9 (2.0–384.0); $P = 0.01$	NR

TABLE 3. Methodological quality of community-based studies included in the all-cause or CVD mortality meta-analysis

Primary author, year (Ref)	Response rate to baseline survey (%)	Length of follow-up adequate	Adequacy of follow-up	Morning blood sampling	Testosterone assay	Exclusion of subjects on treatments that profoundly affect testosterone	Covariates adjusted
Araujo, 2007 (22)[a,b]	52	Yes	Yes	Yes	RIA, extraction	No	Age, BMI, waist circumference, HDL-cholesterol level, systolic BP, race, alcohol consumption, calories expended in physical activity, ever smoking, self-assessed health, self-reported chronic disease
Barrett-Connor, 1988 (23)[b]	82	Yes	Yes	Yes	RIA, extraction	Yes	Age, BP, plasma lipids, blood glucose, cigarette smoking, obesity
Haring, 2010 (33)[a,b]	69	Yes	Yes	No	Platform-based immunoassay	Yes	Age, waist circumference, smoking habits, high-risk alcohol use, physical activity
Khaw, 2007 (24)[a,b]	NR	Yes	Yes	No	Platform-based immunoassay	No	Age, date of visit, BMI, waist-to-hip ratio, systolic BP, cholesterol, history of diabetes, history of hypertension, history of high cholesterol, aspirin use, alcohol intake, cigarette smoking status, physical activity, social class, education level, SHBG, dehydroepiandrosterone sulfate, androstanediol glucuronide
Laughlin, 2008 (31)[a]	82	Yes	Yes	Yes	RIA, extraction	Yes	Age, BMI, waist-to-hip ratio, alcohol use, current smoking, exercise
Lehtonen, 2008 (25)[a]	73	Yes	Yes	Yes	RIA	No	Leptin, CHD, smoking, cardiac insufficiency, Zung depression scale, number of drugs in use, use of alcohol, f-cholesterol, f-triglycerides, f-HDL-cholesterol, insulin 0 h, insulin 2 h, glucose 0 h, glucose 2 h
Menke, 2010 (32)[a,b]	NR	Yes	Yes	Yes	Platform-based immunoassay	No	Age, race/ethnicity, smoking status and pack-years of smoking, household income, education, alcohol consumption, exercise, percent body fat
Shores, 2006 (34)[a]	NR	Yes	Yes	No	Platform-based immunoassay	Yes	Age, BMI, medical morbidity, glucocorticoid and opiate treatment, race, coronary artery disease, COPD, HIV, diabetes, hyperlipidemia, no. of testosterone levels obtained
Smith, 2005 (26)[a,b]	89	Yes	Yes	Yes	RIA, extraction	No	Age, smoking status, adult social class, alcohol consumption, height, FEV_1/height^2, fibrinogen, white blood cell count
Szulc, 2009 (27)[a]	25	Yes	Yes	Yes	RIA, extraction	No	Age, BMI, smoking, physical performance and activity, health status, vitamin D supplementation
Tivesten, 2009 (28)[a]	45	Yes	Yes	No	GC-MS/MS	No	Age, MrOS site, BMI, current smoking, physical activity
Vikan, 2009 (29)[a,b]	NR	Yes	Yes	No	Platform-based immunoassay	Yes	Age, systolic BP, HDL/cholesterol ratio, self-reported diabetes, current smoking, waist/hip ratio

BP, Blood pressure; CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; f, fasting; FEV1, forced expiratory volume in 1 sec; GC-MS/MS, gas chromatography-tandem mass spectrometry; HDL, high-density lipoprotein; MrOS, Osteoporotic Fractures in Men; NR, not reported.

[a] Included in the meta-analysis of all-cause mortality.

[b] Included in the meta-analysis of CVD mortality.

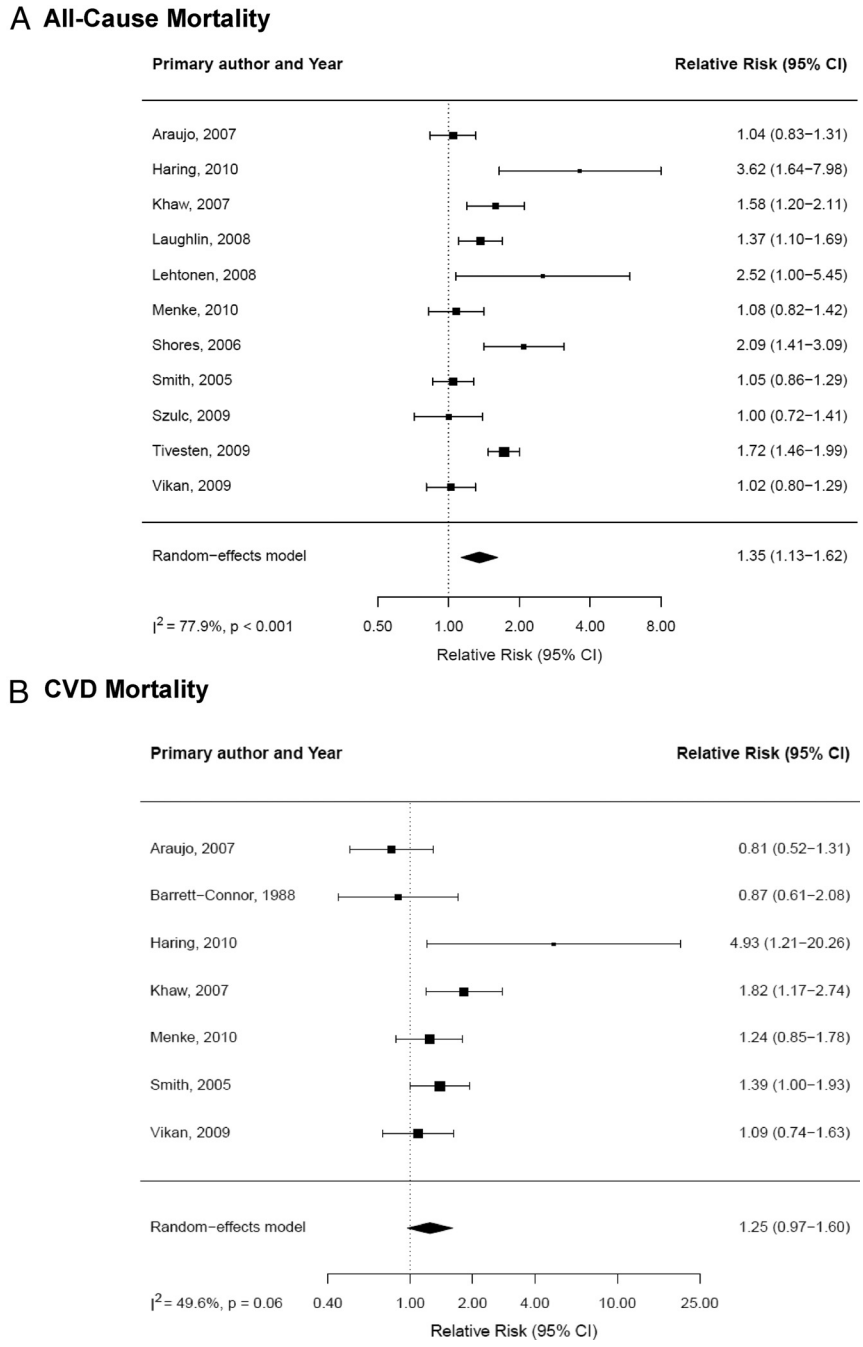

FIG. 2. Association between total testosterone and all-cause (A) and cardiovascular (B) mortality. Each *square* shows the study-specific RR estimate comparing the bottom tertile with the top tertile of the testosterone distribution (the size of the square reflects the study-specific statistical weight, computed as the inverse of the variance), and the *horizontal line* shows the 95% CI on the RR. The *diamond* shows the pooled RR and 95% CI based on random-effects modeling of all studies.

testosterone level ($P = 0.018$), number of years of follow-up ($P = 0.010$), and whether blood samples were collected in the morning ($P = 0.030$). Larger RR were observed in studies that included older (RR = 1.54; 95% CI = 1.28–1.85) *vs.* younger (RR = 1.12; 95% CI = 0.92–1.36) men, subjects with lower (RR = 1.55; 95% CI = 1.28–1.88) *vs.* higher (RR = 1.09; 95% CI = 0.88–1.36) testosterone levels, and subjects who were followed for no more than 9.6 yr (RR = 1.63; 95% CI = 1.32–2.02) *vs.* over 9.6 yr (RR = 1.13; 95% CI = 0.94–1.36) and in studies that did not obtain morning blood samples (RR = 1.61; 95% CI = 1.28–2.03) *vs.* those that did (RR = 1.15; 95% CI = 0.94–1.41). We also examined subjects and study characteristics in relation to the modest degree of heterogeneity in RR for CVD mortality (data not shown). Although none of the factors were statistically significant, those with smaller *P* values for heterogeneity were shorter follow-up period ($P = 0.10$), non-U.S. studies ($P = 0.10$), low baseline total testosterone level ($P = 0.21$), morning blood sample ($P = 0.21$), and high study response rate ($P = 0.33$).

Of the community-based studies, too few reported the association between free (n = 5) or bioavailable (n = 2) testosterone and mortality outcomes to analyze with meta-analysis.

Table 2 describes the nine non-community-based studies that met inclusion criteria for systematic review. Subjects were drawn from diverse patient populations, including patients admitted to intensive care with severe infection, hemodialysis patients, patients attending an andrology clinic for erectile dysfunction, geriatric patients, patients with heart failure, diabetic patients, and patients admitted to a cardiology center with acute myocardial infarction as well as subjects recruited from Medicare files, senior community centers, and apartments. Average follow-up periods ranged from 28 d to up to 4 yr. Of seven studies that presented information on the association between total testosterone and all-cause mortality, two were nonsignificant and the remaining five studies reported significant associations between low testosterone and mortality. Of four studies that presented information on the association between total testosterone and CVD mortality, one was nonsignificant and the remaining three studies reported significant associations between low testosterone and CVD mortality.

TABLE 4. Metaregression of total testosterone and all-cause mortality by subject and study characteristics (n = 11 studies)

Subgroup	No. studies[a]	RR	95% CI	*P* for heterogeneity[b]
Age				0.020
≤60 yr	5	1.12	0.92–1.36	
>60 yr	6	1.54	1.28–1.85	
BMI				0.927
≤26.9 kg/m^2	5	1.47	1.11–1.94	
>26.9 kg/m^2	4	1.44	1.03–2.02	
Prevalence of current smoking				0.256
≤24%	4	1.41	1.12–1.78	
>24%	4	1.15	0.88–1.50	
Baseline total testosterone				0.018
≤487 ng/dl	6	1.55	1.28–1.88	
>487 ng/dl	5	1.09	0.88–1.36	
Length of follow-up period				0.010
≤9.6 yr	5	1.63	1.32–2.02	
>9.6 yr	6	1.13	0.94–1.36	
No. covariates adjusted				0.669
≤8	6	1.31	1.02–1.68	
>8	5	1.43	1.07–1.91	
Geographic location				0.737
Non-U.S.	7	1.40	1.09–1.78	
U.S.	4	1.31	0.97–1.76	
Testosterone assay				0.457
Platform-based	5	1.48	1.11–1.97	
GC-MS/MS or RIA, extraction	6	1.28	1.00–1.64	
Morning blood sampling				0.030
No	5	1.61	1.28–2.03	
Yes	6	1.15	0.94–1.41	
Exclude treatments that profoundly affect testosterone levels				0.331
No	7	1.27	1.01–1.61	
Yes	4	1.55	1.12–2.15	
Study response rate				0.400
≤64%	3	1.24	0.84–1.81	
>64%	4	1.56	1.06–2.31	

GC-MS/MS, gas chromatography-tandem mass spectrometry.

[a] The number of studies within strata of each variable may not sum to 11 due to missing information.

[b] Testing the null hypothesis of no difference in estimated relative risk across subgroup

Discussion

In this systematic review and meta-analysis, we identified 21 studies that met protocol-defined inclusion criteria, 12 of which were eligible for meta-analysis. Although meta-analysis of these community-based studies showed that a decrease of 2.18 SD in total testosterone was associated with a 35 and 25% increased risk of all-cause and CVD mortality, respectively, the observation of considerable between-study heterogeneity (significant for all-cause mortality and borderline significant for CVD mortality) limits the validity of these summary estimates. Factors implicated in study heterogeneity were age, baseline total testosterone, length of the follow-up period, and whether blood samples were collected in the morning.

The results of this study are consistent with a previous systematic review and meta-analysis of endogenous testosterone and CVD morbidity (38) that reported a protective effect of a 1 SD increase in total testosterone (RR = 0.89; 95% CI = 0.83–0.96), significant between-study heterogeneity, and a strong age dependence, with stronger results in older men. Results were also similar regarding the role of a limited set of overlapping study-related factors that failed to contribute to between-study heterogeneity (*i.e.* type of testosterone assay and country of study).

Because concurrent illness is associated with lower sex steroids in men (39), results from clinic-based studies may be biased by the strong possibility that low testosterone is simply an epiphenomenon of concurrent and possibly occult illness. The results of this study indicate that even among relatively well-conducted community-based stud-

ies of generally healthy men, there is significant heterogeneity in the association between endogenous testosterone level and all-cause mortality across studies, which could not be fully accounted for by statistical adjustment. Estimated RR are strongly related to age, baseline total testosterone, length of the follow-up period, and whether blood samples were collected in the morning. Based on these results, one plausible conclusion is that a low testosterone level is simply a risk marker for all-cause mortality. Indeed, given that stronger RR were observed among studies that included older men and among studies that included men with lower baseline total testosterone levels strongly suggests the summary RR may be capturing the influence of some underlying health conditions. Thus, these data would not necessarily be considered supportive of a notion that a large randomized controlled trial should be conducted to test the efficacy of either screening for low testosterone levels or testosterone therapy to prolong life. Nonetheless, the meta-analysis included only 11 studies, which we see as a significant limitation of the current investigation. This limited the ability to investigate potential sources of heterogeneity with multivariable regression models of subgroup effects and the conclusions that could be drawn regarding publication bias. One example of the former was the finding of a weaker association between testosterone and all-cause mortality in studies that collected samples in the morning. We cannot speculate as to the source of this observation, but we examined whether this observation was confounded by age, and heterogeneity was still present with RR estimates unchanged within strata of time of blood sampling in models controlling for age.

The results of the meta-analysis on CVD mortality, on the other hand, indicates no significant ($P = 0.06$) heterogeneity between studies. This must be considered in light of the fact that the statistical test for heterogeneity has relatively low power, particularly when the number of studies included is small. The current literature contains conflicting information from systematic (38) and narrative (40, 41) reviews of endogenous testosterone levels and the development of CVD, which have shown, respectively, a protective effect and no effect of endogenous testosterone levels on CVD.

It is notable that the studies included in this meta-analysis were all observational, and so the data summarized herein are subject to the same limitations associated with this type of study design, primarily selection bias related to nonrandom assignment of exposure (in this case, endogenous testosterone level). A meta-analysis of observational studies cannot overcome these limitations. However, a meta-analysis allows for the provision of a summary measure of association across existing studies and, importantly in this instance, the ability to systematically identify sources of heterogeneity, which may be useful for future researchers.

Implications

The clinical implications of this study are not completely certain but point to the importance of assessing endogenous testosterone levels in middle-aged and elderly men as a potential marker of general health; such assessment should be performed properly, generally in the morning and with a good-quality assay (42). This implication is further supported by recent data indicating that low testosterone levels are strongly related to increasingly common chronic conditions, such as obesity (4, 5), type 2 diabetes mellitus (12), and metabolic syndrome (6) among others. The current study does not address whether screening for low testosterone or treating men with testosterone products would improve clinical outcomes, and the risks and benefits of testosterone therapy remain uncertain (43).

Conclusion

In summary, the current systematic review and meta-analysis has shown that low endogenous testosterone levels are associated with both all-cause and CVD mortality in community-based studies of men. Given the observed between-study heterogeneity, which was partly accounted for by characteristics of the subjects and study methods, it is likely that low testosterone levels are simply a marker of general health.

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