Associations of Testosterone and Related Hormones With All-Cause and Cardiovascular Mortality and Incident Cardiovascular Disease in Men

Individual Participant Data Meta-analyses

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Background: Whether circulating sex hormones modulate mortality and cardiovascular disease (CVD) risk in aging men is controversial.

Purpose: To clarify associations of sex hormones with these outcomes.

Data Sources: Systematic literature review to July 2019, with bridge searches to March 2024.

Study Selection: Prospective cohort studies of community-dwelling men with sex steroids measured using mass spectrometry and at least 5 years of follow-up.

Data Extraction: Independent variables were testosterone, sex hormone–binding globulin (SHBG), luteinizing hormone (LH), dihydrotestosterone (DHT), and estradiol concentrations. Primary outcomes were all-cause mortality, CVD death, and incident CVD events. Covariates included age, body mass index, marital status, alcohol consumption, smoking, physical activity, hypertension, diabetes, creatinine concentration, ratio of total to high-density lipoprotein cholesterol, and lipid medication use.

Data Synthesis: Nine studies provided individual participant data (IPD) (255 830 participant-years). Eleven studies provided summary estimates (n = 24 109). Two-stage random-effects IPD meta-analyses found that men with baseline testosterone concentrations below 7.4 nmol/L (<213 ng/dL), LH concentrations above 10 IU/L, or estradiol concentrations below 5.1 pmol/L had higher all-cause mortality, and those with testosterone concentrations below 5.3 nmol/L (<153 ng/dL) had higher CVD mortality risk. Lower SHBG concentration was associated with lower all-cause mortality (median for quintile 1 [Q1] vs. Q5, 20.6 vs. 68.3 nmol/L; adjusted hazard ratio [HR], 0.85 [95% CI, 0.77 to 0.95]) and lower CVD mortality (adjusted HR, 0.81 [CI, 0.65 to 1.00]). Men with lower baseline DHT concentrations had higher risk for all-cause mortality (median for Q1 vs. Q5, 0.69 vs. 2.45 nmol/L; adjusted HR, 1.19 [CI, 1.08 to 1.30]) and CVD mortality (adjusted HR, 1.29 [CI, 1.03 to 1.61]), and risk also increased with DHT concentrations above 2.45 nmol/L. Men with DHT concentrations below 0.59 nmol/L had increased risk for incident CVD events.

Limitations: Observational study design, heterogeneity among studies, and imputation of missing data.

Conclusion: Men with low testosterone, high LH, or very low estradiol concentrations had increased all-cause mortality. SHBG concentration was positively associated and DHT concentration was nonlinearly associated with all-cause and CVD mortality.

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Older men have lower testosterone concentrations on average than younger or middle-aged men, particularly after age 70 years (1). The decrease in testosterone after age 70 years is accompanied by increased luteinizing hormone (LH) levels, consistent with impaired testicular Leydig cell function (2). Obesity and other medical comorbidities can contribute to lower testosterone concentrations in men by reducing the activity of the hypothalamic–pituitary–testicular (HPT) axis,

even when it is structurally intact (2–4). Given the effects of testosterone on male libido, virilization, and body composition (enhanced muscle mass and reduced fat) (5), an important question has been whether and when lower endogenous testosterone concentrations are related to poorer health outcomes. In the circulation, testosterone is bound to carrier proteins, primarily sex hormone–binding globulin (SHBG), which modulate its availability to tissues (6). Testosterone and SHBG concentrations are related, and men may have low testosterone concentrations in the presence of low SHBG levels without having organic disease affecting the HPT axis (5). Low testosterone in the presence of high LH indicates primary testicular impairment, whereas low testosterone with low or normal LH may be consistent with centrally mediated disease or with suppression of HPT axis function due to obesity and other nongonadal conditions (5, 7). Testosterone is further metabolized to dihydrotestosterone (DHT), which, like testosterone, activates androgen receptors, and to estradiol, which activates estrogen receptors to mediate actions of testosterone in tissues such as bone (8, 9). Understanding the relationship between testosterone and SHBG and these other hormones can inform interpretation of health outcomes associated with differences in hormone concentrations.

Observational studies have reported either null or inverse associations of circulating total testosterone with all-cause and cardiovascular disease (CVD)–related mortality (10). There was a nonlinear association of lower total testosterone with all-cause mortality but no association with CVD deaths or CVD events in men aged 40 to 69 years from the UK Biobank (11, 12). However, the UK Biobank population is relatively healthy, and testosterone was measured by immunoassay rather than mass spectrometry, which provides more accurate and less variable results (13, 14). In studies of older men, higher total testosterone concentrations measured using mass spectrometry were associated with lower risk for CVD events, particularly stroke rather than myocardial infarction (MI) (15, 16). However, a large cardiovascular safety trial in middle-aged and older men with overweight or obesity and cardiovascular risk factors or disease (TRAVERSE [Testosterone Replacement Therapy for Assessment of Long-term Vascular Events and Efficacy Response in Hypogonadal Men]) found that 22 months of testosterone treatment did not increase risk for major cardiovascular events or mortality (17). Therefore, a meta-analysis of prospective cohort studies that measured total testosterone using mass spectrometry in community-dwelling men spanning the age range and different geographic regions could address these contrasting findings. Likewise, mass spectrometry provides greater accuracy and precision than immunoassays for DHT and estradiol, which circulate at much lower concentrations than testosterone in men (1, 13, 14). However, few studies have examined the associations of DHT and estradiol measured using mass spectrometry with mortality and CVD outcomes in men across a range of ages (10).

Our aim was to conduct individual participant data meta-analyses (IPDMAs) of prospective cohort studies of men that measured sex hormones (including total testosterone) using mass spectrometry and had at least 5 years of follow-up for outcomes (18, 19). Our primary hypothesis was that in men, endogenous circulating testosterone concentration is associated with risks for all-cause mortality, CVD death, and incident CVD events. Parallel IPDMAs were conducted for SHBG, LH, DHT, and estradiol to test the secondary hypotheses that any of these variables were associated with these outcomes. Exploratory analyses were conducted with heart failure (HF), MI, and stroke as separate outcomes. Given the relationship between testosterone and SHBG and the implications of high versus low or normal LH concentrations in the setting of lower testosterone concentrations, additional exploratory analyses that jointly considered testosterone and SHBG and testosterone and LH were done.

METHODS

Data Sources and Searches

The AIMS (Androgens In Men Study) protocol was submitted to PROSPERO on 23 July 2019 and registered on 20 November 2019 (CRD42019139668) (18). Eligible studies (data sources) were prospective cohort studies, previously identified in a published systematic review (19), of community-dwelling men with total testosterone concentrations measured using mass spectrometry and at least 5 years of follow-up for CVD events, CVD deaths, all-cause mortality, cancer deaths, cancer diagnoses, cognitive decline, or dementia (Appendix Figure 1, available at Annals.org). Cancer- and dementia-related outcomes will be published separately. The original search included literature to July 2019; a bridge search was conducted to update results from the initial search to May 2023, and a second bridge search was done to update results to March 2024 (2). The bridge searches found 2 studies, neither of which reported 5 or more years of follow-up for outcomes of interest; their authors were not approached for IPD (Supplement Results and Supplement Figure 1, available at Annals.org). This review was done in accordance with the guidance provided in the PRISMA-IPD (PRISMA [Preferred Reporting Items for Systematic reviews and Meta-Analyses] for Individual Patient Data systematic reviews) reporting checklist.

Study Selection

From among 2177 references, we identified 11 eligible studies (19), 9 of which provided IPD-level data (20–32) and 2 of which provided aggregate data (AD) statistics (33, 34). Summary attributes and a flowchart specific to the analyses of all-cause mortality, CVD deaths, and incident CVD events are provided in the Appendix Table (available at Annals.org) and Appendix Figure 1. Within studies, participants

with a history of orchidectomy, those with use of androgens or antiandrogens, those with missing sex hormone measurements, those who were lost to follow-up at an unknown date, and those for whom outcome event status could not be determined were excluded from analyses (Appendix Methods and Appendix Figure 1).

Data Extraction and Quality Assessment

All participants consented to participate in the original cohort studies, and this meta-analysis was approved by the Human Research Ethics Committee of the University of Western Australia. Newcastle-Ottawa quality assessments of the selected studies were performed (Appendix Methods and Supplement Methods, available at Annals.org). Variables for the planned IPDMAs covering sociodemographic, lifestyle, and medical factors; hormone-related exposures; and outcomes were agreed on in advance and published in a protocol paper (Supplement Table 1, available at Annals.org) (18) and requested from each individual study (Appendix Methods). Data sets from individual studies were securely sent, stored in a central repository, and checked, and baseline variables were harmonized (2). Primary health outcomes of interest were all-cause mortality, death caused by CVD, and incident CVD events. Deaths caused by CVD were defined as deaths from MI, cardiac arrest, HF, or stroke, and incident CVD events were defined as occurrence of fatal or nonfatal MI, HF, or stroke. Prospective time-to-event data determined using existing definitions routinely used by each study were requested and supplied as adjudicated event data or derived from supplied IPD using International Classification of Diseases (ICD) codes (Supplement Results and Supplement Table 2, available at Annals.org). Information on numbers of participants, ages, testosterone concentrations, outcome events, and duration of follow-up was tabulated (Appendix Table 1; see also Supplementary Table 6 in the previously published systematic review [19]). Missing data are described in the Appendix Methods, the Supplement Results, and Supplement Tables 3A to 3C (available at Annals.org).

Data Synthesis and Analysis

The IPDMAs summarized relationships between baseline hormone concentrations (total testosterone [primary exposure]; SHBG, LH, DHT, and estradiol [secondary exposures]) and relative risks for all-cause mortality, CVD death, and incident CVD events (outcomes). Random-effects IPDMAs were performed because variations in effect estimates among studies were assumed to be at least partly attributable to differences in local factors (35). The 2-stage approach was adopted to facilitate incorporation of studies with AD (36). This involved fitting the same model to each data set separately (stage 1) and then analyzing the study-specific estimates using a random-effects model (stage 2). The IPDMAs were first conducted using only the 9 IPD-level data sets; then, suitable AD statistics were requested from

the other 2 studies. However, some IPDMAs analyzed fewer studies because studies that did not have IPD for both the outcome and hormone exposure variables were not included (Supplement Table 1). Analyses were performed in R, version 4.0.2 (R Foundation for Statistical Computing).

Two different Cox proportional hazards models were fitted. The minimal model (model 1) included only the terms for hormone exposure and baseline age. The full model (model 2) included the following additional terms: baseline body mass index, marital status, alcohol consumption, smoking status, physical activity, ratio of total to high-density lipoprotein (HDL) cholesterol, hypertension, diabetes, creatinine concentration, and lipid medication use. This set of model predictors encompasses factors that influence testosterone concentrations and potential confounders and were the ones that were included in the initial plan (18) and were available in all 9 IPD-level data sets (Supplement Table 1). In exploratory analyses, additional terms for SHBG category (low, normal, or high) and the interaction between testosterone and SHBG and terms for LH category and the interaction between testosterone and LH were added to model 2. Total–HDL cholesterol ratio was used as an indicator of risk for heart disease (37). For analyses of all-cause mortality, history of cancer at baseline was included as an additional model predictor. For analyses of incident CVD events, participants with a history of CVD were excluded.

Continuous predictors were modeled using restricted cubic splines and used values for centering and knot points that were consistent across studies (Supplement Methods). Substantive model compatible fully conditional specification (SMCFCS) was used to generate 40 imputed versions of each data set, with imputations that were congenial with each IPDMA model (Supplement Methods) (38). The validity of the proportional hazards assumption was assessed for the first imputed data set using per-variable and global tests and Schoenfeld residual plots. Results from fitting each model to each imputed data set in stage 1 were pooled using Rubin's rules before combining in stage 2 (39).

Summary estimates for associations between each outcome and hormone variable are presented in tables and graphically in summary curves and forest plots. Hazard ratios (HRs) and 95% CIs were calculated from each fitted IPDMA model relative to the median of the fifth quintile (Q5 median [reference value]). Full results are presented for IPDMAs of the 9 IPD-level data sets. The relative extent of heterogeneity was quantified using the I^2 statistic (40). Contour-enhanced funnel plots were constructed to explore publication bias. Sensitivity analyses, analyses exploring associations with specific types of CVD events, and analyses that jointly considered testosterone and SHBG and testosterone and LH were also conducted (Appendix Methods and Supplement Methods).

RESULTS

Analysis Population

A detailed description of the analysis population is provided in the Appendix Results and the **Appendix Table**. There were IPD for 20 654 persons for mortality analyses (**Appendix Figure 1**), comprising 255 830 participant-years of risk, with 7241 deaths and 1351 CVD deaths. Aggregate data statistics were provided for 3455 persons, with 1099 deaths and 326 CVD deaths. For CVD event analyses, IPD were available for 12 829 persons and AD were available for another 1956, with 2803 and 326 incident CVD events, respectively.

Testosterone

In an age-adjusted analysis, men with baseline testosterone concentrations at or below the median of Q1 had higher relative risk for all-cause mortality (**Table** [model 1]; **Supplement Figure 2a**, available at Annals. org). In a fully adjusted analysis, the CIs of the HR included 1.0 at all quintile medians (**Table** [model 2]), and only men with low baseline testosterone concentrations (<7.4 nmol/L [<213 ng/dL], where the HR and CI were >1.0) had a higher risk for all-cause mortality (**Figure 1**, *top*).

Age-adjusted CVD mortality risk was higher for men with baseline testosterone concentrations at or below the median of Q1 (**Table** [model 1]; **Supplement Figure 2b**, available at Annals.org). In a fully adjusted analysis, the CIs included 1.0 for all quintile medians (**Table** [model 2]), but the HR and CI were above 1.0 for men with very low testosterone values (<5.3 nmol/L [<153 ng/dL]) (**Figure 1**, *middle*).

Age-adjusted risk for incident CVD events was higher for men with lower baseline testosterone levels (**Table** [model 1]; **Supplement Figure 2c**, available at Annals.org). This was attenuated after all factors were controlled for, with all CIs including 1.0 (**Table** [model 2]; **Figure 1**, *bottom*).

The bounds of the 95% CIs for the I^2 values ranged from 0% to 39.4% for these IPDMAs, demonstrating that although some estimates were imprecise, relative heterogeneity was negligible to moderate (**Table**).

Sex Hormone–Binding Globulin

In age-adjusted and fully adjusted analyses, SHBG was directly associated with all-cause mortality risk for concentrations at and above the Q3 median (37.9 nmol/L) (**Figure 2**, *top*; **Supplement Figure 3a**, available at Annals.org). Age-adjusted HRs and CIs were below 1.0 for Q2 to Q4 medians, and fully adjusted HRs and CIs were below 1.0 for the Q1 to Q4 medians relative to the Q5 median (68.3 nmol/L) (**Table** [model 2]). However, although the age-adjusted risk for all-

cause mortality appeared to be J-shaped (**Supplement Figure 3a**), in both the age-adjusted analysis and the fully adjusted analysis, all-cause mortality risk was lowest for the Q3 median (37.9 nmol/L; HR, 0.83 [95% CI, 0.77 to 0.89]) (**Table** [model 2]; **Figure 2**, *top*).

For associations of SHBG with CVD deaths, 95% CIs of the HRs were broad in age-adjusted and fully adjusted analyses (**Figure 2**, *middle*; **Supplement Figure 3b**, available at Annals.org). This is reflected in the comparison of quintile medians (median for Q1 vs. Q5: fully adjusted HR, 0.81 [CI, 0.65 to 1.00]) (**Table** [model 2]). This increased uncertainty may reflect the smaller number of CVD deaths, as shown on the forest plot for HRs at the Q1 median (20.6 nmol/L) (**Figure 2**, *middle*).

In the age-adjusted analysis, men with low baseline SHBG concentrations had higher risk for incident CVD events (**Supplement Figure 3c**, available at Annals. org). This was attenuated in fully adjusted analyses, with all CIs including 1.0 (**Table** [model 2]; **Figure 2**, *bottom*).

The bounds of the 95% CIs for the I^2 values ranged from 0% to 41.1%, demonstrating that relative heterogeneity was negligible to moderate (**Table**).

Luteinizing Hormone

Summary curves of the HR for all-cause mortality, CVD death, or incident CVD events with LH concentration relative to the Q5 median (10.0 IU/L) were similar in age-adjusted and fully adjusted analyses (**Appendix Figure 2** and **Supplement Figure 4**, available at Annals.org). The CIs included 1.0 at all LH quintile medians for all 3 outcomes (**Table**) and for all LH concentrations for CVD deaths and CVD events (**Appendix Figure 2** and **Supplement Figure 4**). However, men with baseline LH concentrations above 10.0 IU/L (where the fully adjusted HR and the CI were >1.0) had elevated relative risk for all-cause death (**Appendix Figure 2**).

The bounds of the 95% CIs for the I^2 values ranged from 0% to 62.2%, demonstrating that relative heterogeneity was negligible to moderate in these IPDMAs (**Table**).

Dihydrotestosterone

In age-adjusted and fully adjusted analyses, baseline DHT concentration was inversely associated with risks for all-cause and CVD death for concentrations relative to but below the Q5 median (2.45 nmol/L) (**Figure 3**, *top* and *middle*; **Supplement Figure 5**, available at Annals.org). A U-shaped association with all-cause mortality was present, with higher risk for DHT at the Q1 median (0.69 nmol/L) (fully adjusted HR, 1.19 [CI, 1.08 to 1.30]) and for DHT concentrations above the Q5 median (2.45 nmol/L) (**Table** [model 2]; **Figure 3**, *top*). Risk for CVD death was higher at the Q1 median relative to the Q5 median (fully adjusted HR, 1.29 [CI, 1.03 to 1.61]) and for DHT concentrations above the Q5 median (**Table** [model 2]; **Figure 3**, *middle*).

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Table. Summary Estimates of Hazard Ratios for All-Cause Mortality, CVD Death, and Incident CVD Events, by Quintiles of Total Testosterone, SHBG, LH, DHT, and Estradiol Concentrations, and Estimated Relative Heterogeneity*

Outcome	Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)	I^2 (95% CI), %
Total testosterone						
Median concentration						
nmol/L	8.46	11.83	14.68	18.15	24.50	–
ng/dL	244	340	423	523	706	–
Hazard ratio (95% CI)						
All-cause mortality						
Model 1†	1.09 (1.01–1.17)	0.96 (0.88–1.04)	0.93 (0.84–1.03)	0.95 (0.89–1.00)	Reference	0.0 (0.0–30.2)
Model 2‡	1.06 (0.97–1.15)	0.97 (0.90–1.05)	0.96 (0.87–1.05)	0.97 (0.92–1.03)	Reference	0.0 (0.0–35.7)
CVD death						
Model 1†	1.32 (1.06–1.64)	1.17 (0.92–1.49)	1.18 (0.90–1.55)	1.13 (0.96–1.32)	Reference	0.4 (0.0–39.4)
Model 2‡	1.13 (0.86–1.47)	1.05 (0.78–1.40)	1.11 (0.83–1.48)	1.10 (0.95–1.28)	Reference	8.1 (0.0–38.8)
Incident CVD events§						
Model 1†	1.28 (1.14–1.43)	1.13 (1.00–1.28)	1.00 (0.87–1.15)	0.98 (0.90–1.07)	Reference	0.0 (0.0–18.1)
Model 2‡	1.06 (0.92–1.22)	0.99 (0.86–1.13)	0.94 (0.81–1.09)	0.96 (0.88–1.04)	Reference	0.0 (0.0–29.3)
SHBG						
Median concentration, *nmol/L*	20.60	29.80	37.90	48.35	68.30	–
Hazard ratio (95% CI)						
All-cause mortality						
Model 1†	0.94 (0.84–1.05)	0.87 (0.81–0.93)	0.84 (0.77–0.90)	0.88 (0.84–0.93)	Reference	0.0 (0.0–30.8)
Model 2‡	0.85 (0.77–0.95)	0.84 (0.79–0.90)	0.83 (0.77–0.89)	0.88 (0.84–0.93)	Reference	0.0 (0.0–22.0)
CVD death						
Model 1†	0.98 (0.80–1.20)	1.09 (0.85–1.40)	1.10 (0.87–1.39)	1.03 (0.90–1.18)	Reference	10.4 (0.0–41.1)
Model 2‡	0.81 (0.65–1.00)	0.99 (0.78–1.25)	1.06 (0.84–1.34)	1.03 (0.90–1.18)	Reference	0.0 (0.0–37.5)
Incident CVD events§						
Model 1†	1.27 (1.08–1.49)	1.12 (0.98–1.28)	1.07 (0.93–1.22)	1.03 (0.96–1.12)	Reference	0.0 (0.0–36.7)
Model 2‡	0.97 (0.87–1.10)	0.96 (0.86–1.07)	0.96 (0.85–1.08)	0.98 (0.91–1.05)	Reference	0.0 (0.0–25.2)
LH						
Median concentration, *IU/L*	2.19	3.36	4.44	6.00	10.00	–
Hazard ratio (95% CI)						
All-cause mortality						
Model 1†	0.90 (0.73–1.09)	0.85 (0.68–1.06)	0.87 (0.67–1.14)	0.92 (0.76–1.11)	Reference	32.1 (0.0–62.2)
Model 2‡	0.95 (0.79–1.13)	0.90 (0.75–1.07)	0.91 (0.72–1.14)	0.93 (0.79–1.11)	Reference	16.9 (0.0–53.0)
CVD death						
Model 1†	0.88 (0.71–1.09)	0.83 (0.65–1.05)	0.86 (0.60–1.24)	0.92 (0.69–1.21)	Reference	0.0 (0.0–48.1)
Model 2‡	0.85 (0.66–1.10)	0.83 (0.66–1.03)	0.85 (0.65–1.13)	0.91 (0.73–1.13)	Reference	0.0 (0.0–42.5)
Incident CVD events§						
Model 1†	0.89 (0.75–1.07)	0.90 (0.76–1.07)	0.91 (0.76–1.10)	0.94 (0.82–1.08)	Reference	0.0 (0.0–39.5)
Model 2‡	0.92 (0.68–1.24)	0.94 (0.74–1.20)	0.93 (0.74–1.17)	0.95 (0.81–1.11)	Reference	0.0 (0.0–45.2)
DHT						
Median concentration, *nmol/L*	0.69	1.09	1.41	1.78	2.45	–
Hazard ratio (95% CI)						
All-cause mortality						
Model 1†	1.21 (1.11–1.32)	1.09 (1.00–1.20)	1.04 (0.92–1.19)	0.99 (0.93–1.05)	Reference	6.7 (0.0–41.7)
Model 2‡	1.19 (1.08–1.30)	1.10 (1.00–1.21)	1.08 (0.93–1.26)	1.01 (0.93–1.09)	Reference	0.0 (0.0–48.1)
CVD death						
Model 1†	1.40 (1.09–1.79)	1.21 (0.93–1.57)	1.25 (0.93–1.67)	1.05 (0.91–1.22)	Reference	9.5 (0.0–46.0)
Model 2‡	1.29 (1.03–1.61)	1.19 (0.96–1.48)	1.33 (0.97–1.82)	1.09 (0.92–1.27)	Reference	0.0 (0.0–41.1)
Incident CVD events§						
Model 1†	1.34 (1.16–1.54)	1.19 (1.04–1.38)	1.08 (0.92–1.27)	1.02 (0.94–1.11)	Reference	0.0 (0.0–8.4)
Model 2‡	1.18 (0.98–1.41)	1.07 (0.89–1.29)	1.03 (0.85–1.24)	1.01 (0.92–1.10)	Reference	0.0 (0.0–36.4)
Estradiol						
Median concentration, *pmol/L*	40.56	59.86	73.79	90.00	119.12	–
Hazard ratio (95% CI)						
All-cause mortality						
Model 1†	1.03 (0.83–1.29)	0.93 (0.76–1.13)	0.93 (0.79–1.09)	0.96 (0.89–1.03)	Reference	34.6 (0.0–61.4)
Model 2‡	0.98 (0.78–1.25)	0.93 (0.73–1.18)	0.96 (0.80–1.16)	0.98 (0.90–1.06)	Reference	24.1 (0.0–55.5)
CVD death						
Model 1†	1.04 (0.84–1.29)	0.96 (0.72–1.27)	0.88 (0.63–1.22)	0.90 (0.76–1.05)	Reference	23.2 (0.0–54.9)
Model 2‡	1.02 (0.81–1.27)	1.01 (0.76–1.32)	0.98 (0.71–1.35)	0.96 (0.80–1.14)	Reference	28.4 (0.0–58.0)

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Outcome	Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)	I^2 (95% CI), %
Incident CVD events§						
Model 1†	1.04 (0.90–1.19)	0.98 (0.85–1.13)	0.89 (0.69–1.14)	0.96 (0.83–1.10)	Reference	0.0 (0.0–43.1)
Model 2‡	1.06 (0.92–1.22)	0.99 (0.85–1.14)	0.91 (0.71–1.17)	0.98 (0.85–1.12)	Reference	0.0 (0.0–41.9)

CVD = cardiovascular disease; DHT = dihydrotestosterone; LH = luteinizing hormone; Q = quintile; SHBG = sex hormone–binding globulin.
* Hazard ratios were calculated for the medians of testosterone, SHBG, LH, DHT, and estradiol concentrations within each sample quintile (Q1 to Q5) relative to the median for Q5 as calculated from the summary curve. Quintile boundaries as determined from all individual participant data across studies were as follows. Total testosterone: Q1/2, 10.4 nmol/L (300 ng/dL); Q2/3, 13.2 nmol/L (380 ng/dL); Q3/4, 16.3 nmol/L (470 ng/dL); and Q4/5, 20.5 nmol/L (591 ng/dL). SHBG: Q1/2, 25.5 nmol/L; Q2/3, 33.8 nmol/L; Q3/4, 42.6 nmol/L; and Q4/5, 55.7 nmol/L. LH: Q1/2, 2.9 IU/L; Q2/3, 3.9 IU/L; Q3/4, 5.1 IU/L; and Q4/5, 7.2 IU/L. DHT: Q1/2, 0.9 nmol/L; Q2/3, 1.3 nmol/L; Q3/4, 1.6 nmol/L; and Q4/5, 2.0 nmol/L. Estradiol: Q1/2, 51.4 pmol/L; Q2/3, 66.7 pmol/L; Q3/4, 80.4 pmol/L; and Q4/5, 100.0 pmol/L.
† Model 1 included terms for the respective hormones and age.
‡ Model 2 included the model 1 terms plus body mass index, marital or de facto relationship status, education, alcohol consumption, smoking status, physical activity, ratio of total to high-density lipoprotein cholesterol, hypertension, diabetes, creatinine, and use of lipid medications (a proxy for hyperlipidemia). History of cancer was also included as a predictor in analyses of all-cause mortality. Continuous variables (total testosterone, SHBG, LH, DHT, estradiol, age, body mass index, ratio of total to high-density lipoprotein cholesterol, creatinine) were modeled using restricted cubic splines.
§ Participants with a history of CVD were excluded from analyses of incident CVD events.

In the age-adjusted analysis, men with a baseline DHT concentration at or below the Q2 median had increased risk for incident CVD events (**Table** [model 1]; **Supplement Figure 5**). In fully adjusted analyses, this inverse association was attenuated (**Table** [model 2]), with the HR and CI above 1.0 only in men with very low DHT concentrations (<0.59 nmol/L) (**Figure 3**, bottom).

The bounds of the 95% CIs for the I^2 values ranged from 0% to 48.1%, demonstrating that relative heterogeneity was negligible to moderate in these IPDMAs (**Table**).

Estradiol

Summary curves of the HR for all-cause death, CVD death, and incident CVD events with estradiol concentration relative to the Q5 median (119.1 pmol/L) were similar in age-adjusted and fully adjusted analyses (**Appendix Figure 3** and **Supplement Figure 6**, available at Annals.org). The CIs for estradiol quintile medians included 1.0 for all 3 outcomes (**Table**). Only men with very low baseline estradiol concentrations (<5.1 pmol/L, for which the fully adjusted HR and CI were >1.0) had higher risk for all-cause mortality relative to the Q5 median, with no associations for CVD deaths or CVD events in the age-adjusted or fully adjusted analyses (**Appendix Figure 3**; **Supplement Figure 6**).

The bounds of the 95% CIs for the I^2 values ranged from 0% to 61.4%, demonstrating that relative heterogeneity was negligible to moderate in these IPDMAs (**Table**).

Other Analyses

Sensitivity analyses and analyses exploring associations with specific types of CVD events were done (Appendix Results; **Supplement Figures 7 to 17**, available at Annals.org). Estimates of summary HRs from IPDMAs incorporating AD from CHAMP (Concord Health and Ageing in Men Project) and the MrOS (Osteoporotic Fractures in Men) study in Sweden (11 stu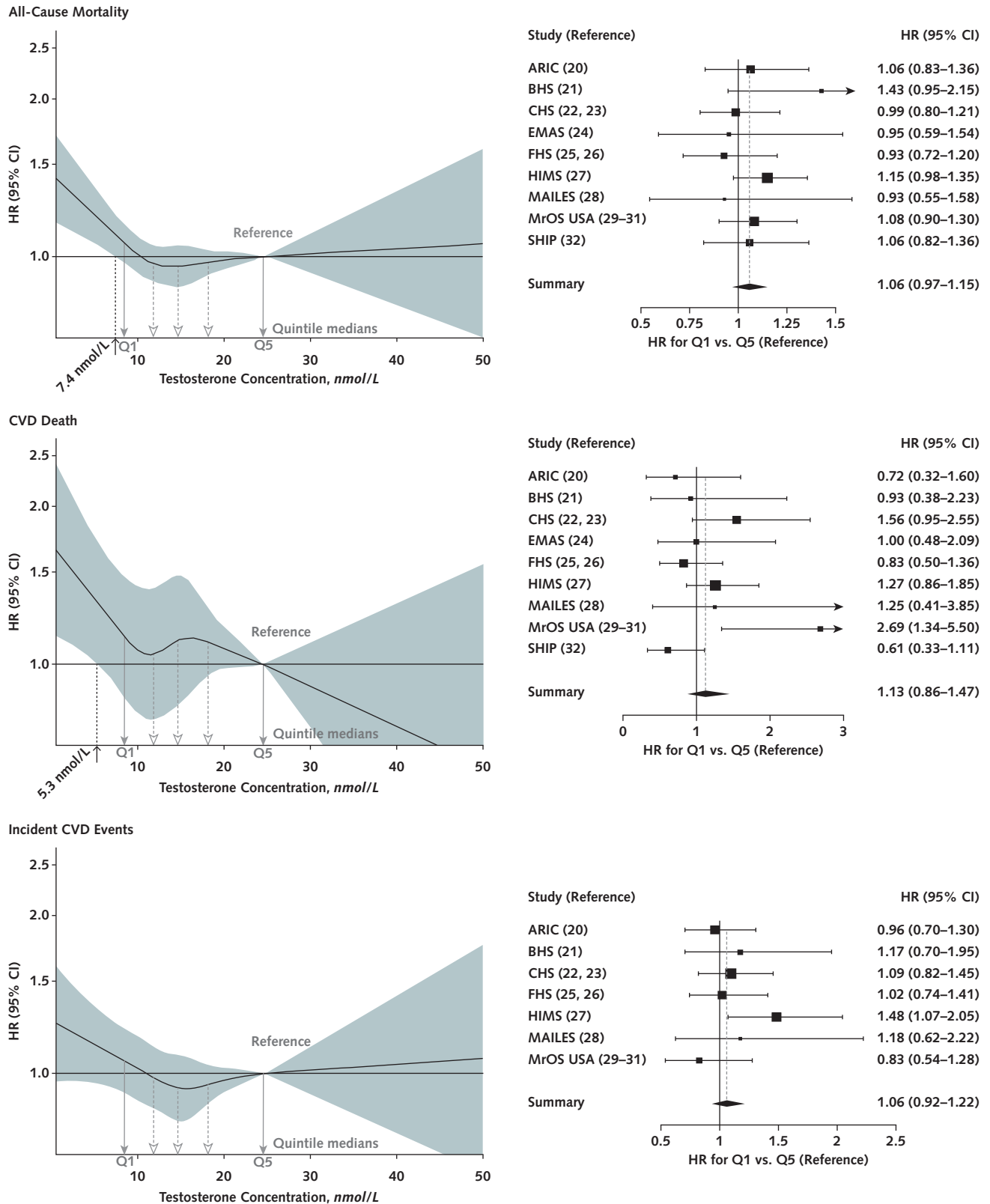dies in total) did not differ substantively from those that used IPD only (9 studies) and had low to negligible heterogeneity, suggesting that the AD from those studies were comparable to others and that availability bias from studies not providing IPD was negligible (**Appendix Figure 4**, available at Annals. org). Furthermore, no important asymmetry was apparent in funnel plots (**Supplement Results** and **Supplement Figure 18**, available at Annals.org). Exploratory analyses indicated that the association of lower testosterone with higher all-cause mortality risk was attenuated for men with lower SHBG concentrations, whereas risk was largely consistent across categories of LH (**Supplement Results**, **Supplement Table 4**, and **Supplement Figure 19**, available at Annals.org).

DISCUSSION

This is, to our knowledge, the first IPDMA of major prospective cohort studies using mass spectrometry sex steroid assays, which clarifies previous inconsistent findings on the influence of sex hormones on key health outcomes in aging men. In fully adjusted analyses, only men with very low total testosterone concentrations had higher risks for all-cause and CVD mortality. A key finding was that men with a testosterone concentration below 7.4 nmol/L (<213 ng/dL) had higher risk for all-cause mortality, regardless of LH concentration. In addition, a higher concentration of circulating SHBG was associated with risk for all-cause mortality and was marginally associated with CVD deaths. Furthermore, the estimated relative risks for all-cause and CVD mortality were elevated in men with low or very high baseline concentrations of DHT. However, only men with very low DHT concentrations had higher risk for incident CVD events. Men with very high LH or very low estradiol concentrations had higher risk for all-cause mortality.

We found that men with low testosterone concentrations (<7.4 nmol/L [<213 ng/dL]) had higher all-cause mortality, and this was consistent in exploratory analyses whether LH was categorized as low, normal, or high. This suggests that the association is with low

Figure 1. Summary curves and forest plots for the association of baseline testosterone concentration with relative risk for all-cause mortality (*top*), CVD death (*middle*), and incident CVD events (*bottom*): model 2.

testosterone itself, whether due to an underlying testicular cause or centrally mediated. Men with even lower testosterone concentrations (<5.3 nmol/L [<153 ng/dL]) had higher risk for CVD death. In an exploratory analysis, lower testosterone concentration was associated with higher risk for stroke, but not MI or HF. Lower testosterone concentrations may predispose men to poorer outcomes via associations with lower muscle mass and greater adiposity and with other adverse cardiovascular risk factors (2, 10). Very low testosterone concentrations might also be associated with other medical comorbidities and hence higher mortality risk (2, 41). By contrast, in other exploratory analyses, men with both low testosterone and normal or high SHBG concentrations had higher all-cause mortality risk, whereas men with low testosterone and low SHBG concentrations had lower risk. Therefore, the combination of low testosterone and low SHBG concentrations seems to be benign, which is consistent with the concept that men with low SHBG may also have low circulating testosterone in the absence of androgen deficiency (5, 7). Our IPDMA resolves previous inconsistent findings from studies using immunoassay (11, 42–51) or mass spectrometry (23, 52–57), which reported either null or inverse associations of testosterone concentration with mortality in men. Similarly, previous studies reported neutral (11, 23, 42–45, 54, 56, 57) or inverse (46, 48, 50) associations of testosterone with CVD mortality in men. By using a large, combined data set of cohorts with testosterone measured using mass spectrometry to obtain more precise estimates, we demonstrate that lower testosterone concentrations are associated with higher all-cause and CVD mortality in a nonlinear fashion.

In our IPDMA, men with higher SHBG concentrations had higher risk for all-cause mortality; this association was linear for SHBG values above the median concentration. There was a marginal association of lower SHBG with lower risk for CVD death. It is possible that higher SHBG concentrations could modulate the bioavailability of testosterone to tissues (6). This concept is supported by our exploratory analysis that found an association between lower testosterone concentrations and higher all-cause mortality when SHBG concentration was normal or high but not when it was low. A direct role for SHBG signaling via receptors on cell surfaces has also been postulated (58, 59). Alternatively, SHBG might influence risk for all-cause and CVD mortality in a manner distinct from its relationship
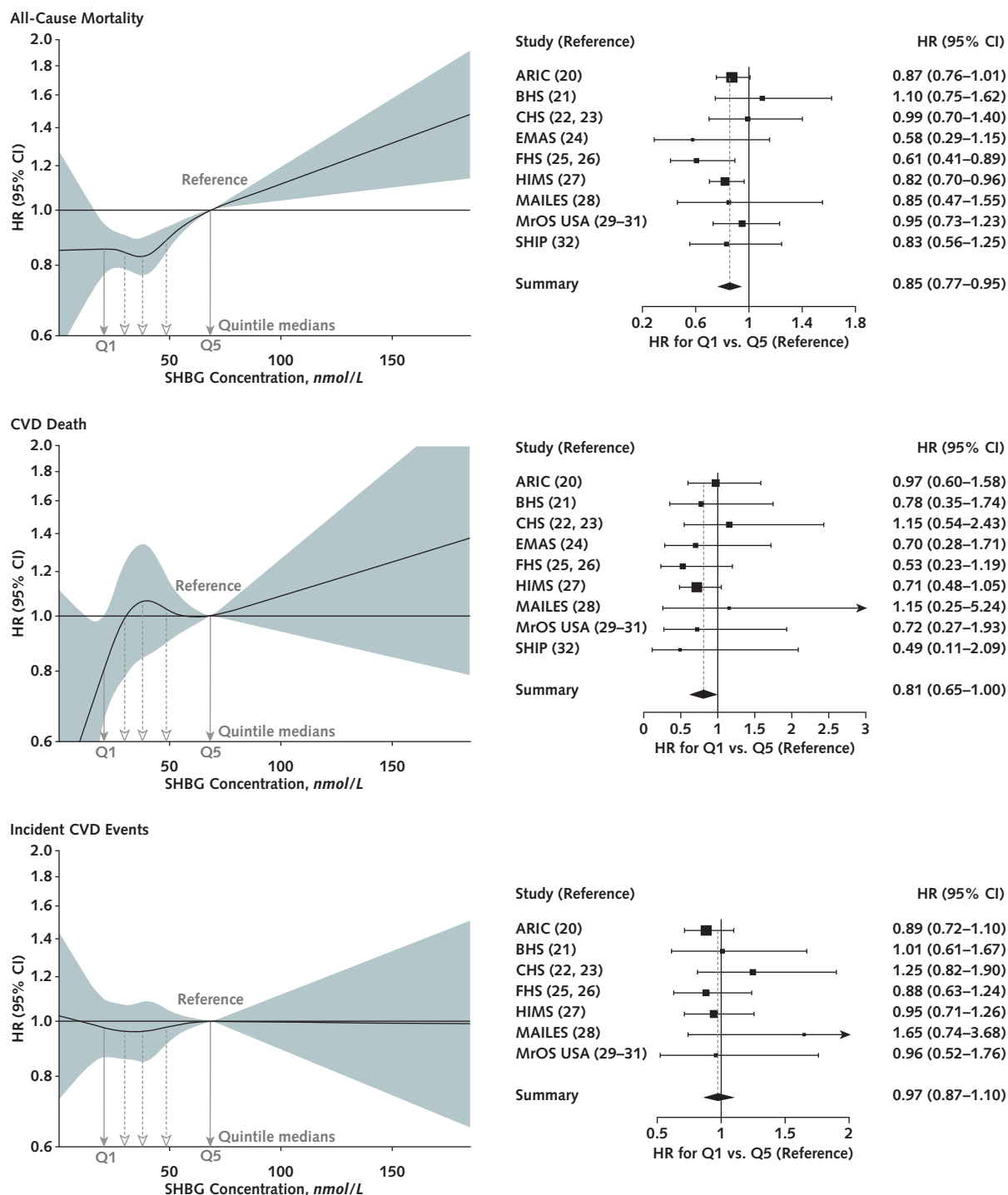
with total testosterone, but further investigation of potential underlying nutritional or metabolic pathways would be needed (60). This IPDMA clarifies results for SHBG where previous studies reported inconsistent findings for all-cause or CVD mortality risk in men (11, 23, 44, 46, 51, 54, 55, 57, 61–63).

Only men with high LH concentrations (as might be consistent with primary hypogonadism) or very low estradiol concentrations (at the margins of the distributions where data were relatively scant) had higher all-cause mortality risk. There was no clear association of LH or estradiol with risk for CVD death. This resolves inconsistent findings from previous studies that reported no association of LH with all-cause mortality (45), higher risk among men with LH in the highest quartile (46), or higher risk among older men with relatively high LH concentrations (61) and from estradiol studies using mass spectrometry that found no association with all-cause mortality (54, 56) or found associations of estradiol in the lowest quartile (55) or decreasing estradiol concentrations with higher all-cause mortality risk (57).

We found that the relationship between DHT and all-cause and CVD mortality risk was consistent with a U-shaped curve. There was increased all-cause mortality risk for men with DHT concentrations in the lowest and second-lowest quintiles and increased CVD mortality risk for the lowest quintile compared with the highest quintile median (2.45 nmol/L) as well as higher risk for both outcomes in men with DHT concentrations above 2.45 nmol/L, although data at these high concentrations were sparse. This clarifies previous inconsistent results from other studies (23, 42, 54, 56, 57). Of note, men with very low DHT had higher risk for incident CVD events; none of the other exposures was related to this outcome. DHT activates androgen receptor signaling, and downstream metabolites of DHT can also activate signaling through other receptors (8), which are potential pathways to modulation of outcomes. Thus, although lower circulating DHT level is a biomarker for poorer health outcomes in men, higher DHT concentrations are not beneficial.
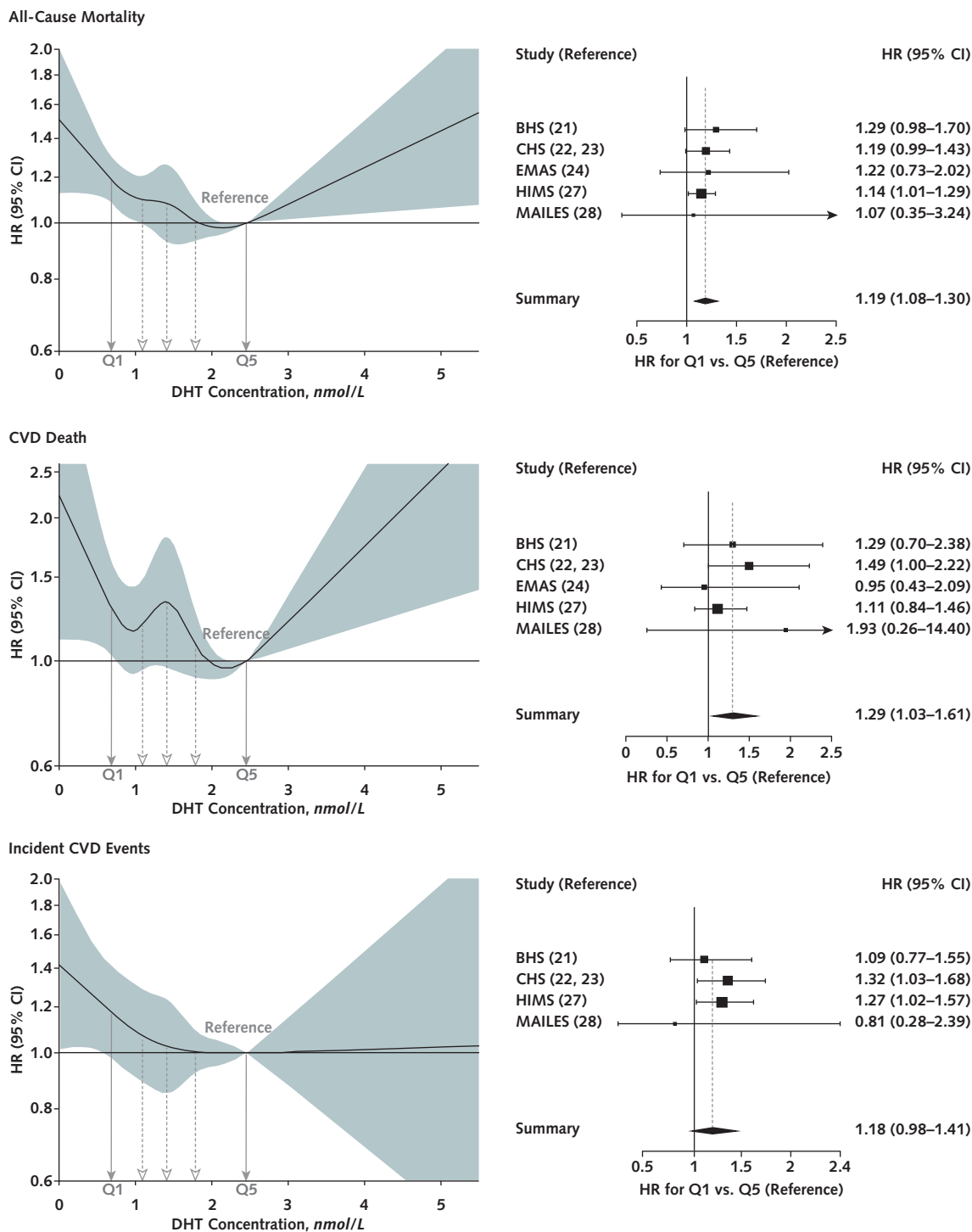
Thus, when examining 3 major sex steroids that activate nuclear transcription factors, we found that only men with low testosterone, low DHT, or very low estradiol concentrations have higher risk for all-cause mortality, and only men with very low testosterone or low DHT concentrations have increased CVD mortality. On the other hand, men with higher concentrations of SHBG,

Figure 2. Summary curves and forest plots for the association of baseline SHBG concentration with relative risk for all-cause mortality (*top*), CVD death (*middle*), and incident CVD events (*bottom*): model 2.

Estimates are controlled for baseline age, body mass index, marital status, alcohol consumption, smoking, physical activity, ratio of total to high-density lipoprotein cholesterol, blood creatinine concentration, lipid medication use, hypertension, and diabetes. Estimates for all-cause mortality are also controlled for history of cancer at baseline. Men with prevalent CVD were excluded from the analysis of incident CVD. ARIC = Atherosclerosis Risk In Communities; BHS = Busselton Health Study; CHS = Cardiovascular Health Study; CVD = cardiovascular disease; EMAS = European Male Ageing Study; FHS = Framingham Heart Study; HIMS = Health In Men Study; HR = hazard ratio; MAILES = Men Androgen Inflammation Lifestyle Environment and Stress; MrOS = Osteoporotic Fractures in Men; Q = quintile; SHBG = sex hormone–binding globulin; SHIP = Study of Health in Pomerania.

Figure 3. Summary curves and forest plots for the association of baseline DHT concentration with relative risk for all-cause mortality (*top*), CVD death (*middle*), and incident CVD events (*bottom*): model 2.

All-Cause Mortality

Study (Reference)	HR (95% CI)
BHS (21)	1.29 (0.98–1.70)
CHS (22, 23)	1.19 (0.99–1.43)
EMAS (24)	1.22 (0.73–2.02)
HIMS (27)	1.14 (1.01–1.29)
MAILES (28)	1.07 (0.35–3.24)
Summary	1.19 (1.08–1.30)

HR for Q1 vs. Q5 (Reference)

CVD Death

Study (Reference)	HR (95% CI)
BHS (21)	1.29 (0.70–2.38)
CHS (22, 23)	1.49 (1.00–2.22)
EMAS (24)	0.95 (0.43–2.09)
HIMS (27)	1.11 (0.84–1.46)
MAILES (28)	1.93 (0.26–14.40)
Summary	1.29 (1.03–1.61)

HR for Q1 vs. Q5 (Reference)

Incident CVD Events

Study (Reference)	HR (95% CI)
BHS (21)	1.09 (0.77–1.55)
CHS (22, 23)	1.32 (1.03–1.68)
HIMS (27)	1.27 (1.02–1.57)
MAILES (28)	0.81 (0.28–2.39)
Summary	1.18 (0.98–1.41)

HR for Q1 vs. Q5 (Reference)

Estimates are controlled for baseline age, body mass index, marital status, alcohol consumption, smoking, physical activity, ratio of total to high-density lipoprotein cholesterol, blood creatinine concentration, lipid medication use, hypertension, and diabetes. Estimates for all-cause mortality are also controlled for history of cancer at baseline. Men with prevalent CVD were excluded from the analysis of incident CVD. BHS = Busselton Health Study; CHS = Cardiovascular Health Study; CVD = cardiovascular disease; DHT = dihydrotestosterone; EMAS = European Male Ageing Study; HIMS = Health In Men Study; HR = hazard ratio; MAILES = Men Androgen Inflammation Lifestyle Environment and Stress; Q = quintile.

which binds all 3 sex steroids, have higher risks for all-cause and CVD mortality. Higher LH concentration, which might be found in men with primary hypogonadism (5, 7), is also associated with higher all-cause mortality.

Strengths of this study include the availability of IPD from 9 prospective cohort studies and AD from 2 other studies, representing prospective data for 24 109 men from 11 major cohort studies (median ages, 49 to 76 years) from Australia, Europe, and North America (20–34). Mass spectrometry was used to measure circulating testosterone, DHT, and estradiol concentrations, providing a more accurate and precise estimate of sex hormone concentrations (13, 14). We conducted prespecified, comprehensive analyses examining 3 key outcomes (all-cause mortality, CVD deaths, and incident CVD events); performed multiple sensitivity analyses; and assessed for publication bias. The size of the combined data set and the statistical approach facilitated robust analyses to resolve different findings from individual studies and to account for potential confounding from cardiovascular risk factors. Limitations of the study include its observational nature, which precluded determination of causation. In bridge searches covering 2019 to 2024, 2 other similarly sized studies were identified that may be eligible for inclusion in future meta-analyses. Sex hormones were assayed in different laboratories at different times. Testosterone is affected by circadian rhythm, and although most studies collected blood samples in the morning, 3 studies did not (2). Validated measures of unbound ("free") testosterone were not available; thus, we presented exploratory analyses of testosterone including SHBG in the model, which provided some insight into outcomes when testosterone concentration was low and SHBG concentration was low, normal, or high. Some variables were recorded differently across studies; these were categorized to enable data to be harmonized. Some cohorts used internal or adjudicated definitions of CVD-related outcomes, and when these were not available, ICD codes were used. We performed analyses to evaluate sensitivity to the inclusion of other variables not featured in the main models, but there may be residual confounding from unmeasured variables, including other chronic diseases that could affect sex hormones or mortality risk, such as depression or thyroid disease. The analyses and conclusions apply to men in the contributing cohorts, who had baseline testosterone concentrations measured using mass spectrometry and were predominantly White and from Australia, Europe, and North America; therefore, our results should be confirmed in studies involving men of different ethnicities from other geographic regions. Nevertheless, findings from this IPDMA represent a broader population than individual studies and provide greater statistical power. Relative heterogeneity in the IPDMAs ranged from negligible to moderate, with 95% CIs for the I^2 values of 0% to 41.1% for analyses of testosterone and SHBG, 0% to 48.1% for DHT, and 0% to 62.2% for LH and estradiol.

In conclusion, we found that the testosterone concentration below which men had higher risk for all-cause mortality was 7.4 nmol/L (213 ng/dL). This adds to information on reference ranges based on distributions of testosterone in selected samples of healthy men (5, 7). Higher SHBG concentrations were associated with higher all-cause mortality, which may be related to its role as the major binding protein for sex steroids in the circulation. We found a U-shaped association of DHT with all-cause and CVD-related mortality risks, which were higher at lower and very high DHT concentrations. Men with very low DHT concentrations also had increased risk for incident CVD events. Further investigation into potential underlying mechanisms for these associations is warranted.

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Reproducible Research Statement: *Study protocol:* The study protocol and systematic review have been published (18, 19). *Statistical code:* Computer code used to generate the results is available on reasonable request (e-mail, bu.yeap@uwa.edu.au). *Data set:* Availability of IPD and AD is governed by ethical approvals of the original cohort studies.

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Author contributions are available at Annals.org.

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APPENDIX: ADDITIONAL DETAILS ON ANALYSES

Methods
Data Extraction
Individual participant data were requested from each individual study after approvals had been granted by the respective institutional review boards (18, 19). Once prepared, IPD were securely sent, stored in a central repository, and checked, and baseline variables from different data sets were harmonized by being converted into a consistent format (categorical variables) or unit of measurement (continuous variables). Primary health outcomes of interest were all-cause mortality, death caused by CVD, and incident CVD events. Deaths caused by CVD were defined as deaths from MI, cardiac arrest, HF, or stroke, and incident CVD events were defined as occurrence of fatal or nonfatal MI, HF, or stroke. Whenever a study routinely used existing definitions for an outcome (such as for adjudicated events), those definitions were used; otherwise, they were derived from supplied IPD using ICD codes (**Supplement Table 2**).

Quality Assessment and Risk of Bias
A description of the literature search and selection of studies, which was conducted by 2 independent investigators with discussion to resolve disagreements, has been published (19). The Newcastle-Ottawa Quality Assessment Scale for Cohort Studies was used to assess selected studies for risk of bias (see Supplementary Table 8 in the previously published systematic review [19]). For the criterion of participant selection (representativeness of the exposed cohort, selection of the nonexposed cohort, ascertainment of exposure, and demonstration that the outcome of interest was not present at the start of the study), the quality of the selected studies ranged from 3 to 4 stars out of a maximum of 4. For the criterion of comparability (based on the study design or analysis), all of the selected studies rated 2 stars (the maximum score). For the criterion of outcome (method of assessment, adequate period of follow-up, adequacy of follow-up), the selected studies rated 2 or 3 stars out of a maximum of 3. Overall, the studies were generally of high quality and had low risk of bias. This reflected the nature of the studies, which were population-based, used an accurate method for measuring testosterone, adjusted for participant age and other risk factors, and had at least 5 years of follow-up for relevant health outcomes (19).

Exclusions
Men with a history of orchidectomy were excluded because we were unable to distinguish those who had unilateral surgery (such as those who had a single testis removed due to testicular cancer or trauma and may or may not have had preserved Leydig cell function from the remaining testis) versus bilateral surgery (such as those who might have had surgical castration for treatment of prostate cancer). Men with a known diagnosis of severe hypogonadism due to hypothalamus–pituitary–gonadal axis pathology would likely have been treated with testosterone, and men reporting use of androgens were excluded. We excluded men using androgens or antiandrogens, as these might have affected circulating testosterone concentrations or interfered with effects of testosterone on the outcomes of interest.

Missing Data
Within each study, the percentage of incomplete cases ranged from 0.5% to 47.2%, and within variables (across all IPD), the percentage ranged from 0% to 6.9% (**Supplement Table 3**). The total number lost to follow-up or with undetermined event status was 419 for mortality analyses and 16 for CVD analyses, representing 2.0% and 0.1% of IPD, respectively, after all other exclusions (**Supplement Tables 3A** to **3C**).

Additional Analyses
Additional analyses explored sensitivity to inclusion of additional predictors not available in all IPD-level data sets, sensitivity to inclusion of AD from the 2 studies that did not supply IPD, sensitivity to extended follow-up of vital status for most but not all EMAS (European Male Ageing Study) participants, associations with specific types of CVD events (HF, MI, and stroke), and whether

associations of testosterone with all-cause mortality risk were influenced by SHBG or LH concentrations (**Supplement Methods**).

Results

Analysis Population

Nine studies provided IPD with follow-up on all-cause and/or CVD deaths (deaths due to MI, cardiac arrest, HF, or stroke) (20–32), and 2 other studies provided AD statistics (33, 34) (**Appendix Table**). After exclusion of men with missing testosterone measurements ($n = 6547$), those with prior orchidectomy ($n = 61$), those using androgens or antiandrogens ($n = 244$), those who were lost to follow-up ($n = 394$), and those with undetermined event status ($n = 25$), there were IPD for 20 654 men for mortality analyses (**Appendix Figure 1**), comprising 255 830 participant-years of risk, with 7241 deaths and 1351 CVD deaths. Aggregate data statistics were provided for 3455 men, with 1099 deaths and 326 CVD deaths. Seven studies provided IPD with follow-up on incident CVD events (occurrence of fatal or nonfatal HF, MI, or stroke). After the same exclusions were applied and men with prior CVD were excluded, there were IPD for 12 829 men for CVD events analyses (**Appendix Figure 1**), comprising 158 445 participant-years of risk and 2803 incident CVD events, and AD for another 1956 men, with 326 CVD events.

Other Analyses

Supplementary analyses controlled for additional covariates that were not available in all of the data sets, with results largely consistent with those of the main analyses (**Supplement Results** and **Supplement Figures 7** to **15**). The estimated risk for HF was higher in men with very low baseline testosterone concentrations (<3.2 nmol/L). The estimated risk for stroke was higher in men with Q1 median testosterone concentrations (8.46 nmol/L) relative to the Q5 median (fully adjusted HR, 1.49 [CI, 1.04 to 2.15]). There was no association of baseline testosterone with risk for MI and no association of SHBG with these outcomes (**Supplement Results** and **Supplement Figures 16** and **17**).

Appendix Table. Summary Attributes: Cross-Sectional Data at Baseline, Numbers of Outcome Events, and Length of Follow-up, by Study

Study (Reference)*	Location	Participants, n	Median Age (IQR), y	Median Testosterone Concentration (IQR), nmol/L	Outcome	Events, n	Median Follow-up (IQR), y
Mortality analyses†							
ARIC (20)	United States	1556	63.0 (58.0–68.0)	13.1 (10.0–16.6)	All-cause	696	20.7 (19.9–21.4)†
					Cardiovascular	70	19.7 (14.9–20.9)
BHS (21)	Australia	2020	49.9 (37.6–64.8)	13.0 (10.1–16.6)	All-cause	476	19.6 (18.9–19.6)
					Cardiovascular	110	19.6 (18.6–19.6)
CHS (22, 23)	United States	1123	76.0 (73.3–79.9)	12.7 (9.5–16.5)	All-cause	997	20.7 (20.5–20.9)
					Cardiovascular	214	12.4 (7.2–18.4)
EMAS (24)	Europe	2445	59.1 (50.3–68.7)	16.1 (12.6–20.3)	All-cause	169	4.3 (4.1–4.6)
					Cardiovascular	69	4.3 (4.1–4.6)
EMAS subset‡	Europe	1742	59.1 (50.4–68.7)	16.5 (12.9–20.7)	All-cause	411	14.0 (13.5–14.2)
FHS (25, 26)	United States	3329	49.0 (39.0–59.0)	20.4 (15.7–26.2)	All-cause	645	16.9 (15.8–19.6)
					Cardiovascular	163	16.6 (15.4–19.2)
HIMS (27)	Australia	4121	76.0 (74.0–79.0)	12.4 (9.5–15.6)	All-cause	2368	13.3 (12.7–14.1)
					Cardiovascular	425	12.5 (8.1–13.3)
MAILES (28)	Australia	1975	55.0 (46.0–64.0)	16.5 (12.8–20.6)	All-cause	138	9.8 (9.4–11.1)
					Cardiovascular	42	9.7 (9.3–11)
MrOS USA (29–31)	United States	1976	73.0 (69.0–77.0)	13.4 (10.3–17.0)	All-cause	1162	17.7 (17.2–18.3)
					Cardiovascular	149	14.7 (8.8–17.5)
SHIP (32)§	Germany	2109	51.0 (37.0–64.0)	15.4 (11.9–19.6)	All-cause	590	19.5 (18.2–20.5)
					Cardiovascular	109	19.0 (9.9–20.1)
CHAMP (33)	Australia	1526	76.0 (72.0–80.0)	14.3 (11.1–18.4)	All-cause	774	11.8 (11.3–12.4)
					Cardiovascular	202	9.3 (8.5–10.1)
MrOS Sweden (34)	Sweden	1929	75.3 (72.8–78.3)	15.4 (11.9–19.3)	All-cause	325	6.5 (5.9–7.1)
					Cardiovascular	124	6.4 (5.8–7.0)
Incident CVD events‖							
ARIC (20)	United States	1502	63.0 (58.0–68.0)	13.1 (10.0–16.7)	CVD	450	19.9 (16.6–21.1)
BHS (21)	Australia	1908	48.7 (36.8–63.2)	13.0 (10.2–16.6)	CVD	303	19.6 (18.7–19.6)
CHS (22, 23)	United States	1123	76.0 (73.3–79.9)	12.7 (9.5–16.5)	CVD	568	14.0 (8.4–20.2)
FHS (25, 26)	United States	3016	47.0 (38.0–57.0)	20.6 (15.9–26.6)	CVD	413	15.9 (13.9–17.4)
HIMS (27)	Australia	2718	76.0 (74.0–78.0)	12.6 (9.7–15.8)	CVD	734	12.7 (9.9–13.5)
MAILES (28)	Australia	1770	54.0 (45.0–63.0)	16.6 (13.0–20.8)	CVD	115	9.8 (9.4–11.0)
MrOS USA (29–31)	United States	792	75.0 (71.0–79.0)	13.6 (10.4–17.3)	CVD	220	10.4 (9.6–10.6)¶
CHAMP (33)	Australia	488	76.0 (72.0–79.0)	15.6 (11.6–19.7)	CVD	114	11.3 (9.4–12.2)
MrOS Sweden (34)	Sweden	1468	75.3 (72.7–78.1)	15.8 (12.4–19.7)	CVD	212	5.5 (4.8–6.0)

ARIC = Atherosclerosis Risk in Communities Study; BHS = Busselton Health Study; CHAMP = Concord Health and Ageing in Men Project; CHS = Cardiovascular Health Study; CVD = cardiovascular disease; EMAS = European Male Ageing Study; FHS = Framingham Heart Study; HIMS = Health In Men Study; IPD = individual participant data; MAILES = Men Androgen Inflammation Lifestyle Environment and Stress study; MrOS = Osteoporotic Fractures in Men; SHIP = Study of Health in Pomerania.
* All studies supplied IPD-level data except CHAMP and MrOS Sweden, which supplied aggregate-level statistics.
† Among men for whom mass spectrometry measurements of testosterone were available after exclusions and losses to follow-up.
‡ Extended follow-up of deaths from any cause was available for a subset of EMAS participants, excluding those from the Manchester and Szeged cohorts.
§ Data are from the SHIP-0 and SHIP-TREND cohorts.
‖ Participants with a history of CVD were excluded from analyses of incident CVD events.
¶ Follow-up of adjudicated cardiovascular events was from the MrOS Sleep Study visit (December 2003 to March 2005) until 28 February 2015, whereas follow-up of deaths in the MrOS USA study was from the initial baseline (March 2000 to April 2002) until August 2019. A small number of participants (*n* = 25) were excluded from mortality analyses due to unadjudicated death status.
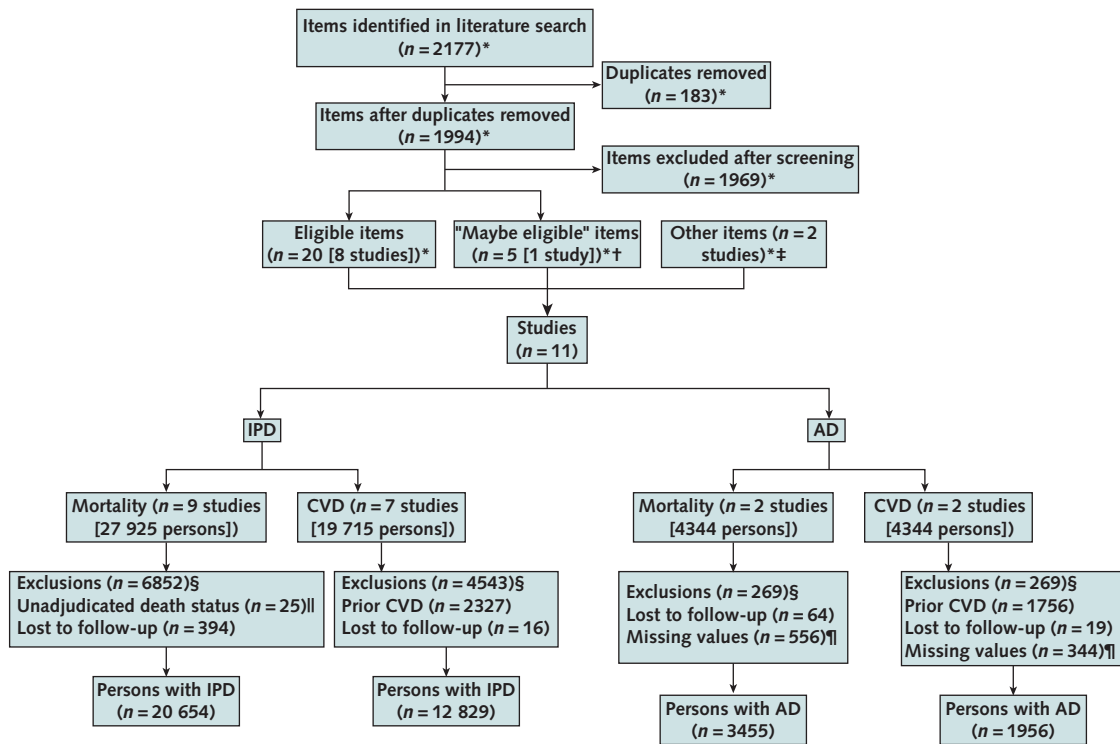
AD = aggregate data; CVD = cardiovascular disease; IPD = individual participant data; MrOS = Osteoporotic Fractures in Men.

* Further details on systematic literature searches and screening and selection of items (journal articles, reports, theses, webpage articles) and a PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flowchart for the systematic review are provided in the article by Marriott and colleagues (19).

† Items identified as "maybe eligible" at completion of systematic screening were further investigated using information external to the systematic review, resulting in identification of 1 additional eligible study with IPD-level data.

‡ Additional studies were identified through known contacts of Marriott and colleagues (18).

§ For analyses of testosterone associations, data were excluded for participants who were missing measurements of total testosterone made using mass spectrometry at baseline, had a history of orchidectomy, were using androgen or antiandrogen medications, or were lost to follow-up.

∥ Participants with unadjudicated death status were excluded from the longer follow-up of mortality outcomes, as at the latest date of data provision by the MrOS USA study.

¶ AD were included from analyses of complete-cases data (missing values excluded).

Appendix Figure 2. Summary curves and forest plots for the association of baseline LH concentration with relative risk for all-cause mortality (*top*), CVD death (*middle*), and incident CVD events (*bottom*): model 2.

All-Cause Mortality

Study (Reference)	HR (95% CI)
BHS (21)	1.33 (0.98–1.81)
EMAS (24)	0.73 (0.40–1.32)
HIMS (27)	0.91 (0.81–1.03)
MAILES (28)	0.41 (0.20–0.84)
MrOS USA (29–31)	0.91 (0.74–1.12)
Summary	0.95 (0.79–1.13)

HR for Q1 vs. Q5 (Reference)

CVD Death

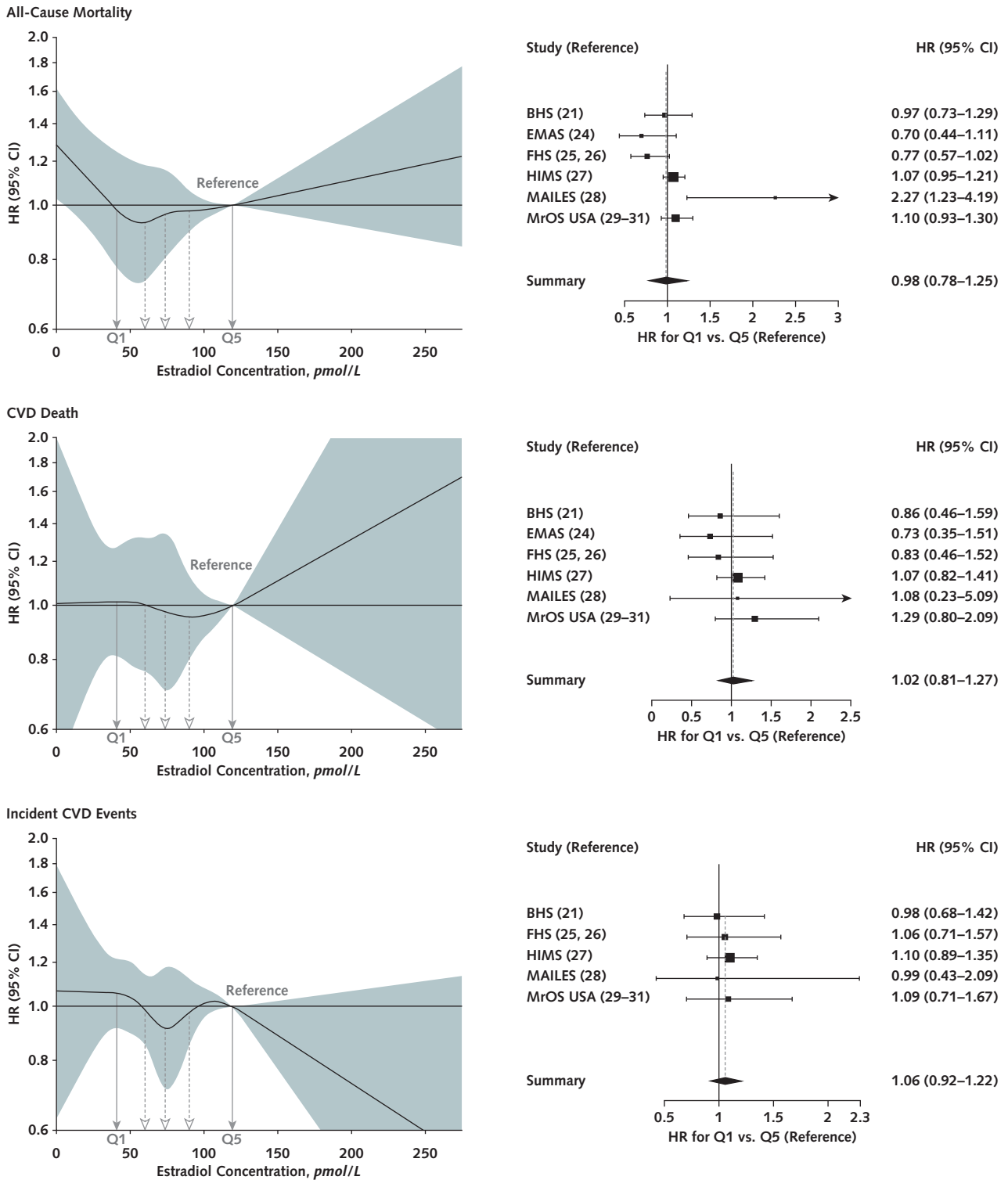| Study (Reference)	HR (95% CI)
BHS (21)	0.88 (0.45–1.74)
EMAS (24)	1.01 (0.44–2.31)
HIMS (27)	0.99 (0.75–1.30)
MAILES (28)	0.35 (0.08–1.62)
MrOS USA (29-31)	0.58 (0.32–1.02)
Summary	0.85 (0.66–1.10)

HR for Q1 vs. Q5 (Reference)

Incident CVD Events

Study (Reference)	HR (95% CI)
BHS (21)	0.97(0.65–1.46)
HIMS (27)	0.94 (0.75–1.16)
MAILES (28)	1.52 (0.85–2.74)
MrOS USA (29-31)	0.55 (0.33–0.93)
Summary	0.92 (0.68–1.24)

HR for Q1 vs. Q5 (Reference)

Estimates are controlled for baseline age, body mass index, marital status, alcohol consumption, smoking, physical activity, ratio of total to high-density lipoprotein cholesterol, blood creatinine concentration, lipid medication use, hypertension, and diabetes. Estimates for all-cause mortality are also controlled for history of cancer at baseline. BHS = Busselton Health Study; CVD = cardiovascular disease; EMAS = European Male Ageing Study; HIMS = Health In Men Study; HR = hazard ratio; LH = luteinizing hormone; MAILES = Men Androgen Inflammation Lifestyle Environment and Stress; MrOS = Osteoporotic Fractures in Men; Q = quintile.

Appendix Figure 3. Summary curves and forest plots for the association of baseline estradiol concentration with relative risk for all-cause mortality (*top*), CVD death (*middle*), and incident CVD events (*bottom*): model 2.
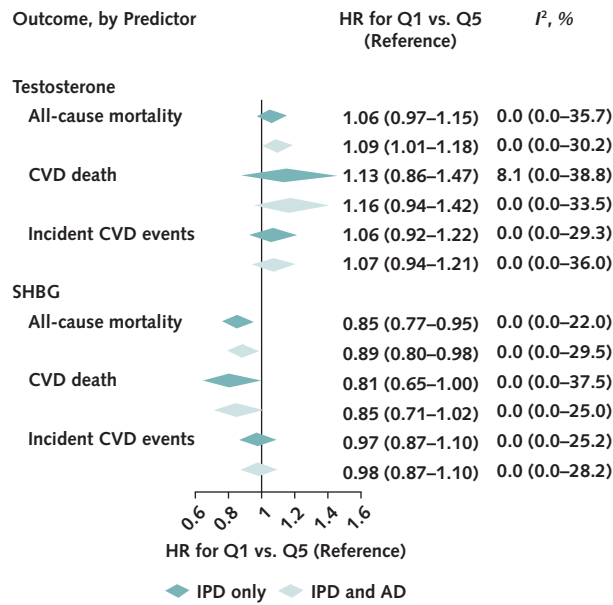

Estimates are controlled for baseline age, body mass index, marital status, alcohol consumption, smoking, physical activity, ratio of total to high-density lipoprotein cholesterol, blood creatinine concentration, lipid medication use, hypertension, and diabetes. Estimates for all-cause mortality are also controlled for history of cancer at baseline. BHS = Busselton Health Study; CVD = cardiovascular disease; EMAS = European Male Ageing Study; FHS = Framingham Heart Study; HIMS = Health In Men Study; HR = hazard ratio; MAILES = Men Androgen Inflammation Lifestyle Environment and Stress; MrOS = Osteoporotic Fractures in Men; Q = quintile.

Appendix Figure 4. Sensitivity of summary estimates (IPD only) to the inclusion of aggregate-level data (IPD and AD) provided by 2 additional studies (CHAMP and MrOS Sweden).

Outcome, by Predictor	HR for Q1 vs. Q5 (Reference)	I², %
Testosterone		
All-cause mortality	1.06 (0.97–1.15)	0.0 (0.0–35.7)
	1.09 (1.01–1.18)	0.0 (0.0–30.2)
CVD death	1.13 (0.86–1.47)	8.1 (0.0–38.8)
	1.16 (0.94–1.42)	0.0 (0.0–33.5)
Incident CVD events	1.06 (0.92–1.22)	0.0 (0.0–29.3)
	1.07 (0.94–1.21)	0.0 (0.0–36.0)
SHBG		
All-cause mortality	0.85 (0.77–0.95)	0.0 (0.0–22.0)
	0.89 (0.80–0.98)	0.0 (0.0–29.5)
CVD death	0.81 (0.65–1.00)	0.0 (0.0–37.5)
	0.85 (0.71–1.02)	0.0 (0.0–25.0)
Incident CVD events	0.97 (0.87–1.10)	0.0 (0.0–25.2)
	0.98 (0.87–1.10)	0.0 (0.0–28.2)

HR for Q1 vs. Q5 (Reference)

◆ IPD only   ◆ IPD and AD

AD = aggregate data; CHAMP = Concord Health and Ageing in Men Project; CVD = cardiovascular disease; HR = hazard ratio; IPD = individual participant data; MrOS = Osteoporotic Fractures in Men; Q = quintile; SHBG = sex hormone–binding globulin.