In Older Men an Optimal Plasma Testosterone Is Associated With Reduced All-Cause Mortality and Higher Dihydrotestosterone With Reduced Ischemic Heart Disease Mortality, While Estradiol Levels Do Not Predict Mortality

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Context: Testosterone (T) levels decline with age and lower T has been associated with increased mortality in aging men. However, the associations of its metabolites, dihydrotestosterone (DHT) and estradiol (E_2), with mortality are poorly defined.

Objective: We assessed associations of T, DHT, and E_2 with all-cause and ischemic heart disease (IHD) mortality in older men.

Participants: Participants were community-dwelling men aged 70 to 89 years who were residing in Perth, Western Australia.

Main Outcome Measures: Plasma total T, DHT, and E_2 were assayed using liquid chromatography-tandem mass spectrometry in early morning samples collected in 2001 to 2004 from 3690 men. Deaths to December 2010 were ascertained by data linkage.

Results: There were 974 deaths (26.4%), including 325 of IHD. Men who died had lower baseline T (12.8 $\pm$ 5.1 vs 13.2 $\pm$ 4.8 nmol/L [mean $\pm$ SD], $P = .013$), DHT (1.4 $\pm$ 0.7 vs 1.5 $\pm$ 0.7 nmol/L, $P = .002$), and E_2 (71.6 $\pm$ 29.3 vs 74.0 $\pm$ 29.0 pmol/L, $P = .022$). After allowance for other risk factors, T and DHT were associated with all-cause mortality (T: quartile [Q] Q2:Q1, adjusted hazard ratio [HR] = 0.82, $P = .033$; Q3:Q1, HR = 0.78, $P = .010$; Q4:Q1, HR = 0.86, $P > .05$; DHT: Q3:Q1, HR = 0.76, $P = .003$; Q4:Q1, HR = 0.84, $P > .05$). Higher DHT was associated with lower IHD mortality (Q3:Q1, HR = 0.58, $P = .002$; Q4:Q1, HR = 0.69, $P = .026$). E_2 was not associated with either all-cause or IHD mortality.

Conclusions: Optimal androgen levels are a biomarker for survival because older men with midrange levels of T and DHT had the lowest death rates from any cause, whereas those with higher DHT had lower IHD mortality. Further investigations of the biological basis for these associations including randomized trials of T supplementation are needed. (***J Clin Endocrinol Metab*** **99: E9–E18, 2014)**

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Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; DHT, dihydrotestosterone; E_2, estradiol; HIMS, Health In Men Study; HR, hazard ratio; IHD, ischemic heart disease; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MMAS, Massachusetts Male Aging Study; MrOS, Swedish Osteoporotic Fractures in Men; Q, quartile; T, testosterone; W, wave; WADLS, Western Australian Data Linkage System; WHR, waist to hip ratio.

Testosterone (T) is the principal androgen produced by the testis under stimulation from pituitary LH. Most circulating T is bound to SHBG and with lesser affinity to albumin, with a small proportion unbound or free (1). Older men have lower levels of T than younger men, but in advanced old age total T levels may be relatively stable, whereas SHBG levels increase, resulting in lower levels of free T (2–5). It is not clear whether low circulating total or free T contributes to disease and death in aging men or whether this is an epiphenomenon related to poor underlying health (6, 7).

Observational studies have associated lower T levels with poorer health outcomes, including incidence of cardiovascular disease (CVD)–related events and mortality (6, 7). However, increased cardiovascular risk is not restricted to men with unequivocally low T levels but may involve men with low to normal T levels, for example, in the lowest quartile (8). In one study of men with low T levels, those given T had lower mortality during follow-up than men not treated with T (9). However, this observational study lacked randomization and had several potential sources of bias or confounding (10). In contrast, a randomized trial of T supplementation in older men with limited mobility was terminated prematurely because of excessive cardiovascular adverse events in the treatment arm (11). Previous comparable studies had failed to report similar findings, although this may have been due to underreporting bias (12). There have been no randomized trials of T therapy with the prespecified endpoints of cardiovascular events or mortality and such necessarily large studies would pose major logistic challenges (4, 13). Consensus clinical guidelines recommend that T therapy be restricted to men with symptoms and signs consistent with androgen deficiency with confirmed low T levels (14). Clarifying associations of T with health outcomes independently of conventional risk factors for ill health would facilitate the design of interventions to ascertain the benefits and risks of T supplementation in aging men.

T regulates male sexual development, virilization, body composition, and bone mineral density (1, 15, 16). The effects of T are modulated in part by its conversion via the intracellular enzyme 5α-reductase to the more potent androgen receptor ligand, dihydrotestosterone (DHT), and by aromatase to estradiol (E_2), a ligand for the estrogen receptors α and β (16, 17). The association of DHT or E_2 levels with ill health in aging men is controversial. Published data are limited for DHT and inconsistent for E_2. An equivocal association of lower DHT with ischemic heart disease (IHD) mortality was reported in one study (18). Although E_2 levels have been positively associated with the progression of carotid intima-media thickness and incident stroke (19, 20), circulating E_2 was negatively associated with mortality in a different study (21). Therefore, additional investigations are needed to clarify whether conversion of T to either metabolite modulates the risk of ill-health during male aging.

In an earlier analysis, we reported that lower calculated free T but not total T, and higher SHBG and LH were associated with mortality in older men (22). In that study, total T was measured by immunoassay, free T was calculated using equilibrium binding equations, and we did not assess DHT or E_2 (5, 22). In the current study, we used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to more accurately assay sex steroids in a population-based cohort of older men (23). We tested the hypothesis that T, DHT, and E_2 would be differentially associated with all-cause and cause-specific mortality in older men.

Subjects and Methods

Study population

The Health In Men Study (HIMS) is a population-based cohort study of community-dwelling older men from Perth, Western Australia, which has been described previously (24). In brief, 12 203 men completed a questionnaire and attended for a physical examination in wave 1 (W1, 1996–1999); 4248 men attended for reassessment and venesection in wave 2 (W2, 2001–2004). Men were almost entirely of Caucasian ethnic origin. The University of Western Australia Human Research Ethics Committee approved the study, and all men gave written informed consent.

Assessment of medical comorbidities

Men were considered to have hypertension if they reported this diagnosis at W1 or W2 or used antihypertensive medication or had blood pressure $\geq$140/90 mm Hg at W2. Dyslipidemia was defined as having fasting high-density lipoprotein of $<$0.9 mmol/L, low-density lipoprotein of $\geq$3.4 mmol/L, triglycerides of $\geq$1.8 mmol/L, or total cholesterol of $\geq$5.5 mmol/L or receiving lipid-lowering therapy at W2. Men diagnosed with diabetes, reporting use of glucose-lowering medication, or having fasting or nonfasting glucose at W2 of $\geq$7 or $\geq$11.1 mmol/L, respectively, were considered to have diabetes (25). Further assessment of morbidity was performed via the Western Australian Data Linkage System (WADLS), which provides electronic linkage to records from death, hospital, and cancer registries and captures admissions to all public and private hospitals in Western Australia (26). Cancer diagnoses were identified from the cancer registry between 1990 and W2. Prevalent CVD was defined as a self-reported history of angina, acute myocardial infarction, stroke, or abdominal aortic aneurysm by questionnaire responses in W1 and W2 or hospital diagnoses of these conditions before W2.

Ascertainment of incident deaths

Occurrence and causes of death were ascertained from WADLS, which contains both the original death certificate and an ICD-10 coded record generated from these data and other

sources by the Australian Bureau of Statistics (26). The primary outcomes were the occurrence of death from any cause and the occurrence of death due to IHD. At the time of linkage, all deaths occurring in Western Australia up to the end of December 2010 had been recorded in WADLS. Surviving men were censored beyond this date.

Laboratory assays

Blood samples were collected between 8:00 and 10:30 AM at W2. Plasma was prepared immediately after phlebotomy and stored at −80°C until assayed. Total T, DHT, and E_2 were quantified within a single LC-MS/MS run without derivatization using atmospheric pressure photoionization in positive mode for androgens and negative mode for estrogens (27), from 200-μL samples as reported previously (28). Precision profiles displayed coefficients of variation <6% for T levels (>0.4 nmol/L), <13% for DHT levels (>0.7 nmol/L), and < 8% for E_2 levels (>25 pmol/L). LH and SHBG had been determined previously by chemiluminescent immunoassays on an Immulite 2000 analyzer (DPC-Biomediq) with coefficients of variation of <7% for both (5). Free T was calculated using empirical formulae, which provide closer concordance with measured free T compared with calculations based on equilibrium binding equations (28, 29).

Statistical analysis

The statistical package Stata (version 12.1; StataCorp) was used. Baseline descriptive data are shown as means ± SD or percentages. Comparisons of means were performed using two-sample *t* tests with equal variances, which are robust for parametric and modestly skewed distributions with sufficiently large sample sizes (30). Nelson-Aalen plots of cumulative mortality according to quartiles of T, calculated free T, DHT, and E_2 were constructed. For the primary longitudinal analysis, Cox proportional hazards regression was performed to assess associations of total T, calculated free T, DHT, and E_2 in quartiles with all-cause and with IHD mortality. Adjustments were made for factors that could plausibly confound any association with mortality. Models were age-adjusted, with subsequent additional adjustment for education, smoking, body mass index (BMI), waist to hip ratio (WHR), then for hypertension, dyslipidemia, diabetes, and creatinine, and finally for history of cancer or existing CVD. The fully adjusted model containing total T was further explored by means of sequential incorporation of SHBG and LH. Calculated free T was not included in any models with T or SHBG because of collinearity. Adjustment for SHBG was performed in the final models involving DHT. Logistic regression was used to graphically plot the probability of dying according to hormone levels. Additional trimmed analyses were performed, excluding men with hormone values in the lowest and highest 1% to ensure that low or high outliers had not skewed the results. A sensitivity analysis was performed, excluding all deaths occurring within the first year of follow-up. A two-tailed *P* value of <0.05 or a 95% confidence interval (CI), which did not overlap 1.0, was considered significant.

Results

Baseline characteristics of the study population

A total of 4248 men provided early morning blood samples in W2. Of these, 4230 had total T, DHT, and E_2 assayed by LC-MS/MS. We excluded from the analyses men receiving androgens (n = 26) or antiandrogen therapy (n = 77) and men with a history of orchidectomy (n = 56) or prostate cancer (n = 381), which left 3690 men.

The follow-up duration was (mean ± SD) 6.7 ± 1.8 years (median, 7.1 years; interquartile range, 6.5–7.8 years) during which 974 men died (26.4%). Of these, 325 deaths were caused by IHD. Characteristics and baseline biochemical variables of the cohort stratified according to survival are shown in Table 1. Men who died were older, were less likely to have completed high school, were more likely to have smoked, and had lower BMI at baseline than surviving men. Men who died were also more likely to have dyslipidemia, CVD, or cancer and higher baseline creatinine levels (Table 1). Baseline T, calculated free T, DHT, and E_2 levels were lower in men who died than in men who survived (Table 1). Men who died of IHD had a higher prevalence of dyslipidemia, diabetes, and CVD, a lower prevalence of cancer, and higher creatinine levels than those who died of non-IHD causes (Supplemental Table 1 published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Cumulative mortality according to quartiles of T, calculated free T, DHT, and E_2

Cumulative mortality was highest for men with total T in the lowest quartile of values (Q1), with the second highest rate seen in men with total T in the highest quartile (Q4) consistent with a nonlinear association (Figure 1A). Men with total T, calculated free T, or DHT in Q3 consistently exhibited the lowest cumulative mortality rates (Figure 1, A–C). Men with E_2 in Q4 had the lowest cumulative mortality rate (Figure 1D).

Hormones and all-cause mortality risk

To accommodate nonlinear associations, hormone levels were analyzed as quartiles. In the fully adjusted analyses, men with total T in the second and third quartiles had significantly lower all-cause mortality than those in the first quartile, whereas the CI for the HR of those in the fourth quartile included 1.00, consistent with a U-shaped association (reference lowest quartile [Q1], Q2:Q1, adjusted HR = 0.82, $P = .033$; Q3:Q1, HR = 0.78, $P = .010$; and Q4:Q1, HR = 0.86, $P = .126$) (Table 2). Similar associations were seen with calculated free T (Q3:Q1, adjusted HR = 0.72, $P = .001$; Q4:Q1, HR = 0.86, $P = .112$) and with DHT (Q3:Q1, adjusted HR = 0.76, $P = .003$; Q4:Q1, HR = 0.84, $P = .059$). Although the crude mortality rate was highest in men with E_2 in the lowest quartile (Q1 28.1% vs Q4 23.5%), there was no difference in risk of death from any cause in the multivariable model (Table 2).

Table 1. Baseline Characteristics of the Study Population

Variable	Alive	Died	*P* Value
No. of participants	2716	974	
Age			
70–74 y	1191 (43.9)	204 (20.9)	
75–79 y	1167 (43.0)	419 (43.0)	<.001
80–84 y	310 (11.4)	273 (28.0)	<.001
≥85	48 (1.8)	78 (8.0)	<.001
Completed high school	1336 (49.2)	435 (44.7)	.016
Smoker			
Never	957 (35.2)	269 (27.6)	
Past	1635 (60.2)	624 (64.1)	<.001
Current	124 (4.6)	80 (8.2)	<.001
BMI ≥25 kg/m^2	1797 (66.2)	577 (60.1)	.001
WHR ≥0.90 cm	2318 (85.3)	813 (84.5)	.532
Hypertension	2091 (77.0)	763 (78.3)	.388
Dyslipidemia	1989 (73.2)	677 (69.5)	.026
Diabetes	402 (14.8)	164 (16.8)	.130
CVD	889 (34.0)	473 (49.9)	<.001
Cancer	238 (8.8)	136 (14.0)	<.001
Creatinine, μmol/L	91.6 ± 28.0	99.0 ± 39.8	<.001
Total T, nmol/L (ng/dL)	13.2 ± 4.8 (380 ± 138)	12.8 ± 5.1 (369 ± 147)	.013
Free T, pmol/L	187.8 ± 53.1	177.5 ± 57.4	<.001
DHT, nmol/L (ng/dL)	1.5 ± 0.7 (44 ± 20)	1.4 ± 0.7 (41 ± 20)	.002
E_2, pmol/L (pg/mL)	74.0 ± 29.0 (20.2 ± 7.9)	71.6 ± 29.3 (19.5 ± 8.0)	.022

Characteristics of the 3690 study participants at baseline, stratified according to whether men had died during the period of follow-up. Data are shown as number (%) for categorical variables or mean ± SD continuous variables. To convert T from nanomoles per liter to nanograms per deciliter, divide by 0.0347, to convert DHT from nanomoles per liter to nanograms per deciliter, divide by 0.0344, and to convert E_2 from picomoles per liter to picograms per milliliter divide by 3.671.

Probability of dying according to hormone levels

Levels of T, calculated free T, and DHT corresponding to values within the third quartile were consistently associated with the lowest risk of dying of any cause (Figure 2, A–C). The CIs were wider for total T, calculated free T, and DHT values above the 75th percentile, and for total T, the CI was consistent with a U-shaped rather than linear association or a threshold for mortality risk.

We repeated the analyses after excluding all men with hormone levels in the lowest or highest 1% of values to ensure that the results were not skewed by low or high outliers (Supplemental Table 2). Compared with men in Q1, men with total or calculated free T or DHT in Q3 (but not Q2 or Q4) had significantly lower all-cause mortality risk.

Models combining T and SHBG, and T and LH in quartiles

When both total T and SHBG were included in the fully adjusted model, total T but not SHBG remained associated with all-cause mortality (Table 3). Compared with men with total T in Q1, those with total T in Q2 to Q4 had lower HRs for death from any cause. When both total T and LH were included in the fully adjusted regression model, having total T in Q3 predicted lower all-cause mortality as did having LH in Q4 (Table 3).

When men with hormone levels in the lowest or highest 1% of values were excluded, total T remained associated with all-cause mortality independently of SHBG (Q3:Q1, adjusted HR = 0.78, 95% CI = 0.62–0.97, $P = .029$). Higher LH was no longer associated with all-cause mortality when total T was included in the model (Q4:Q1, adjusted HR = 1.17, 95% CI = 0.95–1.43, $P = .133$).

Hormones and IHD mortality risk

The associations of total T and calculated free T with IHD mortality resembled those for all-cause mortality with similar adjusted HRs across quartiles but were not statistically significant (Table 4). Higher DHT was associated with reduced IHD mortality, and this association was greater in magnitude than the association of DHT with all-cause mortality and was consistent for all men with DHT above the median value (Q3:Q1, adjusted HR = 0.58, $P = .002$; Q4:Q1, HR = 0.69, $P = .026$). E_2 was not associated with IHD mortality.

In this cohort, DHT and SHBG were closely correlated ($r = 0.48$, $P < .001$). We therefore incorporated SHBG into the final multivariate models involving DHT. In the fully adjusted model incorporating additional adjustment for SHBG, higher DHT was associated with lower all-cause mortality (Q3:Q1, HR = 0.69, 95% CI = 0.57–0.84, $P < .001$; Q4:Q1, HR = 0.73, 95% CI = 0.59–0.89,

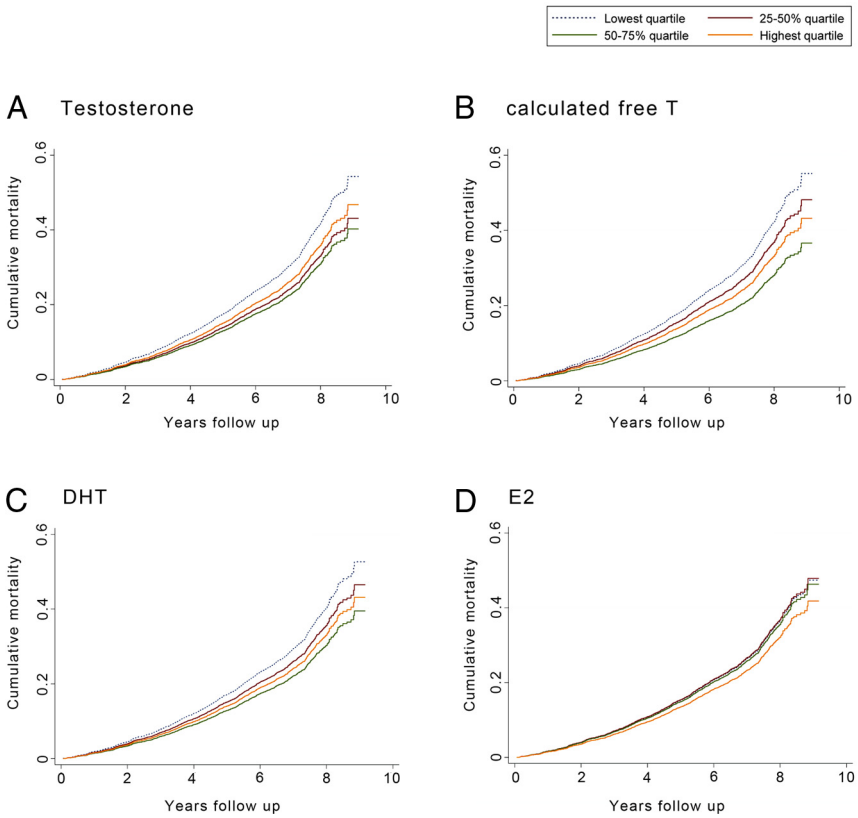

Figure 1. Nelson-Aalen plots showing the cumulative incidence of death from any cause according to quartiles of T (A), calculated free T (B), DHT (C) and estradiol (D) in 3690 community-dwelling men aged 70 to 89 years.

$P = .002$). Higher DHT remained associated with lower IHD mortality independently of SHBG (Q3:Q1, HR = 0.50, 95% CI = 0.35–0.72, $P < .001$; Q4:Q1, HR = 0.57, 95% CI = 0.40–0.81, $P = .002$).

Hormones and non-IHD–related deaths

Either total or calculated free T in Q3 was associated with a lower risk of non-IHD mortality (Supplemental Table 3). Neither DHT nor E_2 was associated with non-IHD mortality.

Sensitivity analyses

We performed additional analyses excluding 57 men who died within 12 months of the date of blood sampling (including 22 from IHD). The associations of total T in Q2 and Q3 and calculated free T in Q3 and DHT in Q3 with lower all-cause mortality were unchanged (Supplemental Table 4). The association of DHT in Q3 and Q4 with lower IHD-related mortality was unchanged (Supplemental Table 5).

Discussion

In older men, having total T levels in the middle two quartiles at baseline predicted reduced incidence of death from any cause, as did having calculated free T or DHT levels in the third quartile. These associations could not be explained by age, overweight, or other risk factors, which were adjusted for systematically in the analyses. A higher DHT level was associated with reduced IHD mortality, but E_2 was not associated with either mortality outcome.

In previous epidemiological studies generally using immunoassays, lower levels of total or free T have been associated with increased mortality without delineating a U-shaped association (7). For example, a case-control study of men aged 42 to 78 years with 825 deaths and 1489 survivors found reduced all-cause mortality in the third and fourth quartiles of total T (31). In a longitudinal study of 794 men aged 50 to 91 years of whom 538 died during 11.8 years of follow-up, total T in the lowest quartile was associated with increased mortality, with a threshold effect (32). A longitudinal analysis of 1114 younger men aged ≥20 years with 206 deaths concluded that lower free T (difference between 90th vs 10th percentile) was associated with all-cause mortality between baseline and 9 years of follow-up (33). There are limited mortality data from studies using mass spectrometry assays for T. In the Swedish Osteoporotic Fractures in Men (MrOS) study of 3104 men aged 69 to 80 years, 383 died during 4.5 years of follow-up. Men with total T in Q2 to Q4 (measured by gas chromatography-mass spectrometry) had lower mortality than those with total T in Q1, with a linear trend (21). Our results challenge the concept that lower T is associated with increased mortality in a linear fashion. Instead, an optimal range of circulating total T corresponding to a range of 9.8 to 15.8 nmol/L (282–455 ng/dL) exists for older men, which predicts survival independent of other risk factors.

Our results differ from those of the Massachusetts Male Aging Study (MMAS) of 1686 men aged 40 to 70 years, in which 395 deaths (101 of IHD) occurred during 15.3 years of follow-up (18). In the MMAS, which used immunoassays for T and DHT and an inaccurate formula to calculate free T, neither T nor DHT was associated with all-cause mortality. Calculated free T was positively associated with deaths of IHD. However, the inverse association of DHT with deaths of IHD was not robust to model selection (18). Our results from a larger cohort of older men using LC-

Table 2. Associations of Sex Hormones in Quartiles With All-Cause Mortality in Older Men

			HR (95% CI)				
Variable	**Range**	**Deaths, %**	**Univariate**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
T, nmol/L							
Q1	0.25–9.82	30.8					
Q2	9.82–12.53	25.1	0.79 (0.67–0.94)	0.84 (0.70–1.00)	0.83 (0.70–0.99)	0.83 (0.69–0.99)	0.82 (0.69–0.98)
Q3	12.56–15.75	23.3	0.74 (0.62–0.89)	0.78 (0.66–0.94)	0.77 (0.65–0.93)	0.80 (0.66–0.95)	0.78 (0.65–0.94)
Q4	15.79–46.50	26.5	0.86 (0.72–1.02)	0.90 (0.76–1.07)	0.86 (0.72–1.03)	0.89 (0.74–1.07)	0.86 (0.72–1.04)
cFT, pmol/L							
Q1	3.54–150.38	31.6					
Q2	150.39–182.63	27.6	0.87 (0.74–1.03)	0.95 (0.80–1.12)	0.94 (0.79–1.12)	0.95 (0.80–1.13)	0.92 (0.77–1.10)
Q3	182.66–216.34	21.3	0.66 (0.55–0.80)	0.74 (0.61–0.88)	0.72 (0.60–0.87)	0.72 (0.60–0.87)	0.72 (0.60–0.87)
Q4	216.34–698.95	24.4	0.78 (0.66–0.94)	0.88 (0.74–1.06)	0.85 (0.70–1.02)	0.87 (0.72–1.05)	0.86 (0.71–1.04)
DHT, nmol/L							
Q1	0.09–0.92	30.9					
Q2	0.93–1.34	27.0	0.88 (0.74–1.05)	0.90 (0.75–1.06)	0.88 (0.74–1.05)	0.89 (0.75–1.06)	0.87 (0.73–1.04)
Q3	1.34–1.83	22.6	0.75 (0.63–0.90)	0.78 (0.65–0.93)	0.76 (0.64–0.92)	0.77 (0.64–0.92)	0.76 (0.63–0.91)
Q4	1.83–7.20	24.2	0.82 (0.69–0.98)	0.84 (0.71–1.01)	0.83 (0.69–1.00)	0.84 (0.70–1.02)	0.84 (0.69–1.01)
E_2, pmol/L							
Q1	2.26–53.60	28.1					
Q2	53.96–70.12	27.6	1.01 (0.85–1.20)	1.02 (0.86–1.21)	1.03 (0.86–1.22)	1.06 (0.89–1.26)	1.09 (0.91–1.30)
Q3	70.24–89.94	26.1	0.98 (0.82–1.16)	1.01 (0.85–1.21)	1.02 (0.86–1.22)	1.04 (0.87–1.24)	1.02 (0.85–1.22)
Q4	90.03–237.88	23.5	0.88 (0.74–1.06)	0.97 (0.81–1.16)	0.97 (0.81–1.17)	0.98 (0.82–1.18)	1.00 (0.83–1.20)

Proportional hazards regression of T, calculated free T (cFT), DHT, and E_2 modeled as quartiles for the outcome of all-cause mortality. Model 1: adjusted for age. Model 2: adjustment as in model 1 and for education, smoking, BMI, and WHR. Model 3: adjustment as in model 2 and for hypertension, dyslipidemia, diabetes, and creatinine. Model 4: adjustment as in model 3 and for prevalent CVD and cancer. To convert T from nanomoles per liter to nanograms per deciliter, divide by 0.0347, to convert DHT from nanomoles per liter to nanograms per deciliter, divide by 0.0344, and to convert E_2 from picomoles per liter to picograms per milliliter divide by 3.671.

MS/MS for assays of T and DHT indicate that total and accurately calculated free T and DHT had comparable and consistent associations with all-cause mortality. Importantly, in our study, a plasma DHT above the median value (≥1.34 nmol/L [39 ng/dL]) was unequivocally associated with lower IHD mortality risk. Of note, higher plasma DHT was associated with lower all-cause and lower IHD mortality independent of SHBG levels.

There are several factors that might explain the contrast between our results and those of previous studies. The HIMS cohort comprises men aged 70 years and older, whereas other studies have included middle-aged (18, 31, 32) or even younger men (33). It is possible that the biological associations of T and DHT with outcomes such as all-cause or IHD mortality might be accentuated across the transition from middle to older age. In addition, the power of Cox regression models is influenced by the number of outcome events, and we observed 974 deaths (325 of IHD) in our cohort. Therefore, we would expect to define associations with these outcomes more robustly than studies with lower numbers of outcome events. Finally, greater precision from the assay of T and DHT by LC-MS/MS and use of an accurate empirical formula to calculate free T might have improved our ability to characterize their associations with all-cause and IHD mortality in older men.

Previous studies reported that higher E_2 levels (measured by immunoassay) were associated with progression of carotid intima-media thickness and with incident stroke (19, 20). The MrOS study in Sweden reported that free E_2 (calculated from total E_2 measured by RIA using an unvalidated formula) was positively associated with peripheral arterial disease (34). In contrast, the subsequent analysis from the MrOS study found that men with low total E_2 (measured by gas chromatography-mass spectrometry) had higher mortality, with the highest mortality rate seen in men with both total T and E_2 in the lowest quartile of values (21). However, multivariable adjustment was limited to age, MrOS site, BMI, physical activity, and current smoking. Our results in a larger cohort of older men with more outcome events and comprehensive adjustment for potential confounders indicate that E_2 is not associated with all-cause or IHD mortality. This is consistent with previous reports in which plasma E_2 was not associated with prevalent CVD or intermittent claudication (28, 35) and with a recent meta-analysis that failed to establish any association of E_2 with CVD-related outcomes (36). Therefore, the effects of E_2 on the vasculature may be more limited than those in other tissues such as bone (16, 17).

Strengths of this study include the analysis of a large cohort of community-dwelling older men, the longitudinal nature of the study with follow-up during which a large number of outcome events occurred, assay of T, DHT, and E_2 using LC-MS/MS with an accurate validated formula to calculate free T, and the systematic adjustment in the statistical analyses for a range of risk factors. Our results were robust to exclusion of low and high outliers for hormone levels and to exclusion of deaths within the first year, which makes reverse causality less likely. Follow-up of men in the cohort is virtually complete, because the

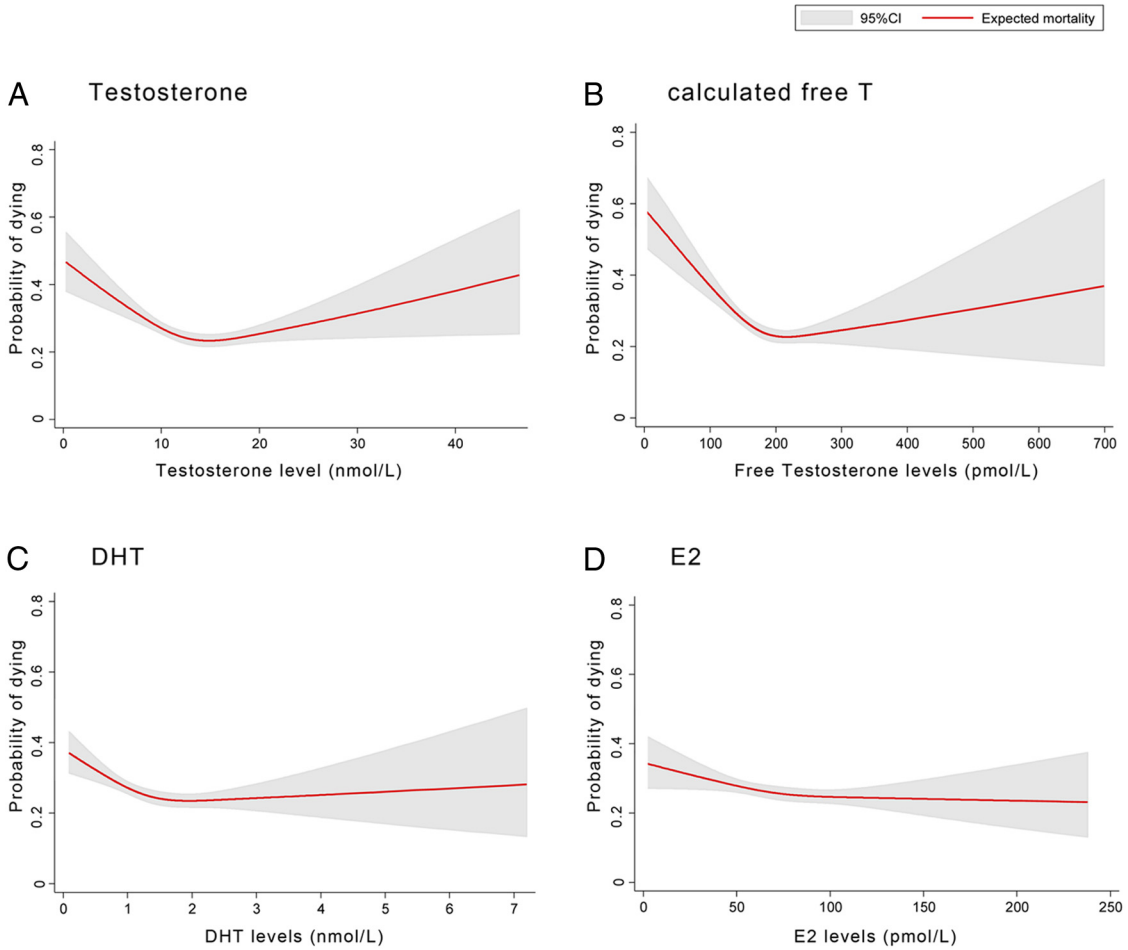

Figure 2. Probability of dying from any cause according to plasma levels of T (A), calculated free T (B), DHT (C) and estradiol (D) in 3690 community-dwelling men aged 70 to 89 years.

WADLS captures mortality data for the entire state of Western Australia, and few men in this age group migrate interstate or overseas (26). We acknowledge several limitations of this study. HIMS is an observational study, limiting our ability to infer causation. We could not distinguish between the effects of comorbidities or their treatments as the use of medication for hypertension, hypercholesterolemia, and diabetes was incorporated into the classification of men with these conditions. Although medical comorbidities were adjusted for in the analyses, we cannot exclude the possibility that the cause of death was related to the presence or absence of treatments for

Table 3. Associations of T and SHBG, and T and LH With All-Cause Mortality in Older Men

A			B		
Variable	**HR (95% CI): Fully Adjusted Model**	***P* Value**	**Variable**	**HR (95% CI): Fully Adjusted Model**	***P* Value**
T, nmol/L			T, nmol/L		
Q1			Q1		
Q2	0.81 (0.68–0.98)	.031	Q2	0.84 (0.70–1.01)	.061
Q3	0.75 (0.61–0.92)	.005	Q3	0.81 (0.67–0.97)	.026
Q4	0.77 (0.61–0.97)	.025	Q4	0.89 (0.73–1.07)	.219
SHBG, nmol/L			LH, IU/L		
Q1			Q1		
Q2	1.00 (0.82–1.23)	.975	Q2	1.06 (0.88–1.29)	.532
Q3	1.05 (0.85–1.30)	.670	Q3	0.90 (0.74–1.10)	.302
Q4	1.23 (0.97–1.57)	.080	Q4	1.22 (1.02–1.47)	.033

Proportional hazards regression models including both T and SHBG (A) and T and LH (B) modeled as quartiles for the outcome of all-cause mortality. HRs (95% CI) are presented for models incorporating hormones as shown, with adjustment for age, education, smoking, BMI, WHR, hypertension, dyslipidemia, diabetes, creatinine, and prevalent CVD and cancer.

Table 4. Associations of Sex Hormones in Quartiles With IHD Mortality in Older Men

			HR (95% CI)				
Variable	**Range**	**Deaths, %**	**Univariate**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
T, nmol/L							
Q1	0.25–9.82	13.4					
Q2	9.82–12.53	10.8	0.79 (0.59–1.06)	0.85 (0.63–1.14)	0.84 (0.63–1.13)	0.86 (0.64–1.16)	0.82 (0.61–1.11)
Q3	12.56–15.75	9.4	0.70 (0.52–0.94)	0.74 (0.55–1.00)	0.75 (0.55–1.02)	0.81 (0.59–1.11)	0.79 (0.58–1.09)
Q4	15.79–46.50	9.2	0.68 (0.50–0.93)	0.72 (0.53–0.98)	0.72 (0.52–0.99)	0.81 (0.58–1.12)	0.79 (0.56–1.11)
cFT, pmol/L							
Q1	3.54–150.38	13.7					
Q2	150.39–182.55	12.6	0.93 (0.70–1.24)	1.05 (0.79–1.39)	1.04 (0.78–1.38)	1.08 (0.81–1.44)	0.99 (0.74–1.33)
Q3	182.66–216.34	8.3	0.61 (0.44–0.84)	0.69 (0.51–0.95)	0.70 (0.50–0.96)	0.71 (0.51–0.99)	0.72 (0.51–1.00)
Q4	216.43–698.95	8	0.59 (0.43–0.82)	0.70 (0.50–0.97)	0.69 (0.49–0.97)	0.77 (0.54–1.08)	0.79 (0.56–1.11)
DHT, nmol/L							
Q1	0.09–0.92	14.5					
Q2	0.93–1.34	11.8	0.83 (0.62–1.09)	0.86 (0.65–1.13)	0.84 (0.63–1.12)	0.89 (0.66–1.18)	0.86 (0.64–1.14)
Q3	1.34–1.83	7.7	0.54 (0.40–0.75)	0.58 (0.42–0.79)	0.58 (0.42–0.80)	0.59 (0.42–0.81)	0.58 (0.42–0.82)
Q4	1.83–7.20	8.5	0.61 (0.44–0.83)	0.64 (0.47–0.87)	0.64 (0.46–0.88)	0.69 (0.50–0.96)	0.69 (0.50–0.96)
E_2, pmol/L							
Q1	2.26–53.60	11					
Q2	53.96–70.12	12	1.12 (0.83–1.52)	1.12 (0.83–1.51)	1.11 (0.82–1.50)	1.19 (0.88–1.62)	1.22 (0.89–1.67)
Q3	70.24–89.94	10.9	1.04 (0.77–1.41)	1.08 (0.80–1.47)	1.08 (0.79–1.46)	1.15 (0.84–1.57)	1.10 (0.80–1.51)
Q4	90.31–237.88	8.9	0.87 (0.63–1.19)	0.96 (0.70–1.33)	0.98 (0.71–1.35)	1.04 (0.75–1.45)	1.09 (0.78–1.51)

Proportional hazards regression of T, calculated free T (cFT), DHT, and E_2 modeled as quartiles for the outcome of IHD mortality. Model 1: adjusted for age. Model 2: adjustment as in model 1 and for education, smoking, BMI, and WHR. Model 3: adjustment as in model 2 and for hypertension, dyslipidemia, diabetes, and creatinine. Model 4: adjustment as in model 3 and for prevalent CVD and cancer. To convert T from nanomoles per liter to nanograms per deciliter, divide by 0.0347, DHT from nanomoles per liter to nanograms per deciliter, divide by 0.0344, E_2 from picomoles per liter to picograms per deciliter divide by 3.671.

comorbidities. Men who provided blood samples were drawn from a larger group seen previously (24); thus, a "healthy survivor" effect may be present, which could make our findings more applicable to healthier older men. The study involved a single blood sample for hormone assays, albeit drawn early in the morning to minimize confounding from circadian variation. Although serial blood samples were not available for hormone assays, sampling at a single time point offers a reasonable estimate of T and DHT levels (37). The men in the HIMS are almost entirely Caucasian, so we cannot draw conclusions for men of other ethnic origins.

Our previous analysis, based on immunoassay for T and performed after 605 of the men had died, showed no association of total T with all-cause mortality, whereas lower calculated free T, higher SHBG, and higher LH were associated (22). In contrast, in the current study based on LC-MS/MS, associations of total and calculated free T were similar and consistent with DHT with regard to all-cause mortality. Differences in study outcomes could be due to the nonspecificity of T results from immunoassay especially at the lower circulating levels of men who died during the study time frame or the greater number of deaths analyzed in the current study. Furthermore, provided that total T is measured accurately, calculation of free T by any formula offers no improvement in risk stratification (28). Lower total T rather than higher SHBG or higher LH levels was associated with mortality in combined regression models, reinforcing the predictive value of T based on LC-MS/MS. DHT possesses a greater binding affinity for SHBG than T (38), yet we found associations of higher DHT with lower all-cause and lower IHD mortality to be independent of SHBG, consistent with the observations for total T.

A U-shaped association of total T with all-cause mortality might be explained by contrasting the effects of lower and higher circulating T. Reduced androgen exposure could contribute to mortality risk via several pathways ranging from altered body composition, reduced bone strength, frailty, modulation of cardiovascular risk factors, impairment of vascular function, or effects on angiogenesis and neovascularization (4, 8, 39, 40). The observation that higher DHT was associated with reduced IHD mortality but not with non-IHD mortality is more consistent with a protective influence of androgens against IHD. T supplementation in men with coronary artery disease has been shown in small studies to protect against exercise-induced myocardial ischemia (for example, Ref. 41). Exogenous T undergoes conversion to DHT; therefore, the levels of both androgens would be increased after T supplementation (42). Conversely, high levels of T might be undesirable because T therapy can result in raised hematocrit and decreased high-density lipoprotein cholesterol levels and cardiovascular adverse events in older men with limited mobility (11, 13).

In conclusion, optimal circulating total T is a robust biomarker for survival in aging men. However, higher DHT levels are associated with reduced IHD mortality, consistent with a possible cardioprotective influence of androgen exposure. Further investigations of the biolog-

ical basis for these biomarkers including randomized trials of T treatment are needed to clarify their prognostic utility and assess whether interventions that modulate circulating T or DHT might improve longevity or reduce the risk of CVD events in aging men.

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