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Journal of the American Heart Association

ORIGINAL RESEARCH

Circulating Androgen Concentrations and Risk of Incident Heart Failure in Older Men: The Cardiovascular Health Study

Joyce N. Njoroge, MD; William Tressel, MS; Mary L. Biggs, PhD; Alvin M. Matsumoto, MD; Nicholas L. Smith, PhD; Emily Rosenberg, MD; Calvin H. Hirsch, MD; John S. Gottdiener, MD; Kenneth J. Mukamal, MD, MPH; Jorge R. Kizer, MD, MSc

BACKGROUND: Circulating androgen concentrations in men decline with age and have been linked to diabetes and atherosclerotic cardiovascular disease (ASCVD). A similar relationship has been reported for low total testosterone and incident heart failure (HF) but remains unstudied for free testosterone or the more potent androgen dihydrotestosterone (DHT). We hypothesized that total/free testosterone are inversely related, sex hormone–binding globulin is positively related, and total/free DHT bear a U-shaped relationship with incident HF.

METHODS AND RESULTS: In a sample of men from the CHS (Cardiovascular Health Study) without atherosclerotic cardiovascular disease or HF, serum testosterone and DHT concentrations were measured by liquid chromatography–tandem mass spectrometry, and sex hormone–binding globulin by immunoassay. Free testosterone or DHT was calculated from total testosterone or total DHT, sex hormone–binding globulin, and albumin. We used Cox regression to estimate relative risks of HF after adjustment for potential confounders. In 1061 men (aged 76±5 years) followed for a median of 9.6 years, there were 368 HF events. After adjustment, lower calculated free testosterone was significantly associated with higher risk of HF (hazard ratio [HR], 1.14 [95% CI, 1.01–1.28]). Risk estimates for total testosterone (HR, 1.12 [95% CI, 0.99–1.26]), total DHT (HR, 1.10 [95% CI, 0.97–1.24]), calculated free dihydrotestosterone (HR, 1.09 [95% CI, 0.97–1.23]), and sex hormone–binding globulin (HR, 1.07 [95% CI, 0.95–1.21]) were directionally similar but not statistically significant.

CONCLUSIONS: Calculated free testosterone was inversely associated with incident HF, suggesting a contribution of testosterone deficiency to HF incidence among older men. Additional research is necessary to determine whether testosterone replacement therapy might be an effective strategy to lower HF risk in older men.

Key Words: aging ■ heart failure ■ men ■ sex hormones ■ testosterone

Heart failure (HF) is a foremost public health priority, affecting ≈6 million people in the United States[1] and continuing to increase in prevalence apace with rising life expectancy across the world.[2] HF disproportionately afflicts and is a leading cause of hospitalization in older adults.[1] Its 2 major subtypes, HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF), affect elders in roughly equal proportions.[3] Although considerable progress has been made in defining the pathogenesis of HF in older adults—particularly in the case of HFrEF, for which various effective therapies are available—understanding of the disorder and its subtypes remains incomplete, and prognosis continues to be poor.[3]

Among older men in particular, aging-related decline in testosterone levels has attracted interest as a potential contributor to deterioration in health status and the development of various disorders, including

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CLINICAL PERSPECTIVE

What Is New?

- We found a significant association between low calculated free testosterone and new-onset heart failure (HF) after adjustment for traditional risk factors in community-dwelling older men, but this was not the case for total testosterone, total dihydrotestosterone, or calculated free dihydrotestosterone.
- In secondary analyses, the inverse relationship between calculated free testosterone and HF was documented for HF with reduced ejection fraction but not preserved ejection fraction.

What Are the Clinical Implications?

- These findings support the use of calculated free testosterone over total testosterone for evaluating androgen deficiency and attendant HF risk in advanced old age, but this will require study in separate cohorts.
- Our results suggest that testosterone repletion could lower HF incidence in older men, a premise that warrants testing in clinical trials.

Nonstandard Abbreviations and Acronyms

cfDHT	calculated free dihydrotestosterone
cfT	calculated free testosterone
CHS	Cardiovascular Health Study
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
SHBG	sex hormone–binding globulin
TRAVERSE	Study to Evaluate the Effect of Testosterone Replacement Therapy on the Incidence of Major Adverse Cardiovascular Events and Efficacy Measures in Hypogonadal Men

cardiovascular disease (CVD).[4] Although circulating levels of total testosterone were not associated with incident CVD[5] or HF[6] in middle-aged men, an inverse relationship was documented with both atherosclerotic CVD (ASCVD)[7] and HF[8] in older men. Existing studies on the relationship between circulating androgens and HF risk were subject to several limitations. Available studies primarily relied on immunoassays for testosterone measurement, which have lower accuracy and precision than gold-standard liquid chromatography–tandem mass spectrometry that are standardized by the Centers for Disease Control and Prevention.[9] Moreover, serum testosterone is predominantly bound to sex hormone–binding globulin (SHBG) or albumin, and therefore its biological activity on tissues is affected by concentrations of these carriers.[10] Only 1% to 4% of circulating testosterone is unbound or free and biologically active,[10] yet levels of free testosterone have not been evaluated as it pertains to the development of HF. This is particularly important with advancing age because, in contrast to the decline in circulating total testosterone concentrations, SHBG levels rise with age, further compounding the free testosterone deficit.[11] Finally, no study to date has examined the relationship of dihydrotestosterone, a more potent androgen generated from conversion of testosterone by 5α-reductase,[12] with incident HF, such that the specific contributions of total or free dihydrotestosterone to HF risk remains unknown.

We previously investigated the relationships of circulating total and free testosterone and dihydrotestosterone measured by state-of-the-art liquid chromatography–tandem mass spectrometry, as well as SHBG measured by fluoroimmunoassay, with incident ASCVD[13] and atrial fibrillation[14] in a sample of older men from the CHS (Cardiovascular Health Study). The only significant findings detected were for total and calculated free dihydrotestosterone (cfDHT), which showed similar curvilinear relationships with both ASCVD and atrial fibrillation. Here, we leveraged this same cohort to evaluate the relationships of these analytes with incident HF and its subtypes. We tested the hypotheses that, after adjustment for potential confounders, incident HF would have inverse associations with total and free testosterone, positive with SHBG, and curvilinear with total and free dihydrotestosterone in this sample of male adults late in life.

METHODS

Population

CHS is a population-based, longitudinal study of CVD and its determinants in older adults recruited at clinical sites in California, North Carolina, Maryland, and Pennsylvania.[15] The first cohort of participants, enrolled between 1989 and 1990, included 5201 men and women, with a second cohort of 687 predominantly Black participants enrolled between 1992 and 1993. Participants underwent annual in-person examinations until 1999, and again in 2005. Clinic visits included assessments for medical history, physical examination, phlebotomy and laboratory testing, and diagnostic tests. These visits alternated with, or were replaced by, semiannual telephone contacts during which similar verbal assessments were conducted.

For the present ancillary study, a subset of 1128 men who attended the 1994 to 1995 examination and were

free of CVD (coronary heart disease [CHD], stroke, and HF) were selected for androgen measurement. This enrollment period was chosen on the basis of sample availability as previously described.[14] After exclusion of participants with missing covariates (n=67), there were 1061 men available for inclusion in the analytic sample.

This study received approval from the institutional review boards of participating institutions. All CHS participants provided written informed consent before enrollment. Requests for data access by qualified researchers may be sent to CHS at chsdata@uw.edu.

Hormone Measurements

Serum specimens collected at the 1994 to 1995 examination were stored at –70°C at the CHS Core Laboratory (Burlington, Vermont). Fasting was not required, and timing of collection was not tracked, but specimens were typically obtained during morning hours. Frozen specimens were sent to an experienced research laboratory (author A.M.M.) in 2010 for performance of hormone assays. Total testosterone and dihydrotestosterone serum concentration measurements were conducted using liquid chromatography–tandem mass spectrometry.[16] All assays were performed in duplicate with values subsequently averaged. The assay for total testosterone was certified by the Centers for Disease Control and Prevention Hormone Standardization Program over concentrations spanning the lower limit of detection of 1.0 ng/dL to an upper value of 2000 ng/dL. Intra- and interassay coefficients of variation were 4.9% and 5.1%, respectively. The dihydrotestosterone assay had a lower limit of detection of 0.02 ng/mL, with intra- and interassay coefficients of variation of 5.9% and 6.2%, respectively. SHBG was measured by a time-resolved fluoroimmunoassay (Delfia, Perkin Elmer, Norton, OH) with a lower limit of detection of 0.5 nmol/L and intra- and interassay coefficients of variation of 1.4% and 6.6% at 31 nmol/L. Free testosterone was calculated from total testosterone, SHBG, and albumin using the Vermeulen method,[17] and free dihydrotestosterone was calculated using the Mazer method.[18]

HF Ascertainment

The primary outcome was incident HF, and secondary outcomes were the HF subtypes HFrEF and HFpEF. Surveillance and ascertainment of HF events has been described previously.[19] All potential HF events were adjudicated by the CHS Events Committee, which performed a review of relevant hospital and outpatient medical records. Confirmation was based on documentation of an HF diagnosis by a physician, confirmatory symptoms (dyspnea, fatigue, orthopnea, paroxysmal nocturnal dyspnea) or signs (edema, rales, S3 gallop, displaced left ventricular impulse), pharmacological therapy for HF, or supportive findings on diagnostic testing, including cardiomegaly and pulmonary edema on chest X-ray or echocardiographic findings. Medical records were also reviewed for determination of HF subtype as HFrEF or HFpEF on the basis of imaging assessment of left ventricular function surrounding the event. HFrEF was defined as left ventricular ejection fraction <50% and HFpEF as left ventricular ejection fraction ≥50% when such imaging information was available; HF cases were unclassified otherwise. Ascertainment of HF events was through June 2015.

Covariates

Covariate information was collected during the 1994 to 1995 examination or, when missing, carried forward from previous examinations. Specifically, height, high-density lipoprotein cholesterol, and NT-proBNP (N-terminal pro-B-type natriuretic peptide) were not measured at the 1994 to 1995 visit and were obtained from the 1992 to 1993 examination. Demographic information and lifestyle factors were assessed by self-report. Smoking was defined by >100 cigarettes smoked, and classified as current when occurring in the past 30 days and ever otherwise. Heavy alcohol use indicated consumption of >14 drinks/wk. Physical activity was described by blocks walked per week. Height, weight, and blood pressure were measured using standardized protocols. Laboratory testing included blood glucose, total and high-density lipoprotein cholesterol, cystatin C, C-reactive protein, and NT-proBNP, as previously described.[20] Diabetes was defined as fasting glucose >126 mg/dL, nonfasting glucose >200 mg/dL, and/or use of diabetes therapy. Estimated glomerular filtration rate was calculated as $76.7 \times \text{cystatinC}^{-1.19}$. Methods for ascertainment of incident CVD events have been described.[19] CHD was defined as myocardial infarction or percutaneous or surgical revascularization. Atrial fibrillation or flutter was determined by presence on ECG or by discharge diagnosis codes.[21]

Statistical Analysis

Baseline characteristics were described using standard summary statistics. Follow-up time was calculated as the interval from baseline to the earliest date of HF diagnosis, death, loss to follow-up, or end of observation. Secondary analyses of HFrEF and HFpEF were censored on the date of alternative or unclassified HF events when these were earliest.

The relationships of total and calculated free testosterone (cfT), cfDHT, and SHBG with HF or its subtypes were assessed with Cox proportional hazards models. Hazard ratios (HRs) were reported per SD decrement in continuous levels of androgen measures. The

proportional hazards assumption was tested using Schoenfeld residuals, which revealed no meaningful violations. To account for potential confounders, we fit 2 sequential models: model 1 (minimal model), adjusting for age and Black race; and model 2 (main model), additionally adjusting for body mass index, smoking history, heavy alcohol use, physical activity, systolic blood pressure, antihypertensive medication use, total and high-density lipoprotein cholesterol, and estimated glomerular filtration rate. We examined additional models adjusting for diabetes, atrial fibrillation, or time-varying CHD to evaluate the impact of putative factors in the causal pathway. In sensitivity analyses, we restricted the sample to participants without a history of prostate cancer and those without HF by NT-proBNP levels, defined using the HF rule-out threshold of NT-proBNP<300 pg/mL.[22]

We examined the functional forms of the associations of androgens and SHBG with HF by fitting versions of model 1 with restricted cubic splines, with knots at percentiles determined using Akaike's information criterion, selected from among models with 0 to 9 evenly spaced knots and 3 to 7 knots at Harrell's specified locations.[23] We used a likelihood ratio test to compare the fit of each cubic spline model to that of a linear model.

All analyses report 2-sided *P* values, with the significance level set at 0.05. We did not perform correction for multiple testing since the androgen exposure measures are correlated. Statistical analyses were performed with R statistical software version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics of the study sample, both overall and by quartiles of total testosterone are presented in Table 1, while those for cFT are provided in Table S1. Overall, lower total testosterone and cfT quartiles were associated with Black race; lower physical activity and high-density lipoprotein cholesterol; and higher body mass index, systolic blood pressure, antihypertensive medication use, diabetes, and NT-proBNP; and history of prostate cancer.

There were 368 incident HF events over a median follow-up of 9.6 (maximum, 21.1) years. Of these, 111 (30.2%) were HFrEF, 113 (30.7%) were HFpEF, and 144 (39.1%) could not be classified.

Plots depicting the functional forms for the associations of total testosterone and cfT, total dihydrotestosterone and cfDHT, and SHBG with incident HF are presented in Figure 1**.** No meaningful departures from linearity were observed for total T, cfT, cfDHT, or SHBG. While the test for nonlinearity for the total dihydrotestosterone-HF association was significant (*P*=0.017), the corresponding plot suggests that the association is approximately linear in the range of total dihydrotestosterone values containing the bulk of the distribution. In light of the uncertainty in the form of the association at the extreme values of total dihydrotestosterone, and for ease of interpretation, we chose to present HR estimates from the linear model for total dihydrotestosterone and incident HF.

The risk estimates per SD decrement in level of each of the 5 exposures are given in Figure 2. In the minimally adjusted model (model 1), all exposure measures were inversely associated with incident HF, with the exception of cfDHT. After further adjustment in the main model (model 2), however, only cfT remained statistically significant. Every SD decrement of cfT was associated with a 14% (95% CI, 1%–28%) higher risk of HF. This association persisted after additional adjustment for putative causal intermediates, including prevalent diabetes, atrial fibrillation, and time-varying CHD. When total testosterone and total dihydrotestosterone each were entered in the main model together with SHBG, the risk estimates (per SD decrement) were modestly changed, but those for SHBG were more substantially attenuated, as assessed by their regression coefficients (not shown). Corresponding HRs follow: total testosterone, HR, 1.10 per SD (95% CI, 0.97–1.26) and SHBG, HR per SD, 1.03 (95% CI, 0.91–1.17); total dihydrotestosterone, HR per SD, 1.08 (95% CI, 0.95–1.23) and SHBG, HR per SD, 1.05 (95% CI, 0.92–1.19).

Results were not materially altered in sensitivity analyses limited to participants without a prior history of prostate cancer (n=971). Evaluation of the subset with available NT-proBNP levels (n=911), however, showed that the risk estimate for cfT (per SD decrement) from the main model (HR per SD, 1.11 [95% CI, 0.98–1.27]) was meaningfully attenuated after removal of participants (n=135) with NT-proBNP ≥300 pg/mL (HR per SD, 1.05 [95% CI, 0.91–1.21]). Of these participants with NT-proBNP ≥300 pg/mL, n=15 exhibited levels diagnostic of clinical HF[22] (n=9 participants aged ≤75 years with NT-proBNP ≥900 pg/mL; n=6 aged >75 years with NT-proBNP ≥1800 pg/mL).

A secondary analysis of 224 cases of HF with subtype classification was performed, excluding the 144 cases without EF data. The relationships of androgen measures with HF subtypes are presented in Table 2. Both total testosterone and cfT were inversely associated with incident HFrEF in the minimally adjusted model, and cfT remained significantly associated after main model adjustment with a 26% (95% CI, 1%–57%) higher risk of HFrEF (per SD decrement). Neither SHBG nor total dihydrotestosterone or cfDHT were significantly associated with incident HFrEF. There

Table 1. Baseline Characteristics*, Both Overall and by Quartile of Total Testosterone Concentration

Variable	Overall n=1061	Quartile 1 [2, 275 ng/dL] n=267	Quartile 2 (275, 367 ng/dL] n=271	Quartile 3 (367, 476 ng/dL] n=268	Quartile 4 (476, 1490 ng/dL] n=266
Age, y	76.4 (5.1)	77.1 (5.7)	75.8 (4.7)	76.0 (4.5)	76.6 (5.2)
Race, Black, n (%)	157 (14.8)	51 (19.1)	33 (12.2)	37 (13.8)	38 (14.3)
BMI, kg/m^2	26.7 (3.7)	27.8 (4.0)	27.1 (3.4)	26.5 (3.3)	25.2 (3.4)
Smoking, n (%)					
Never	251 (23.7)	73 (27.3)	58 (21.4)	57 (21.3)	66 (24.8)
Former	697 (65.7)	167 (62.5)	185 (68.3)	180 (67.2)	172 (64.7)
Current	113 (10.7)	27 (10.1)	28 (10.3)	31 (11.6)	28 (10.5)
Heavy alcohol use, n (%)	94 (8.9)	22 (8.2)	28 (10.3)	28 (10.4)	17 (6.4)
Physical activity, blocks/wk	51.8 (68.7)	43.1 (57.5)	49.2 (69.2)	54.6 (72.4)	60.7 (72.8)
Systolic BP, mm Hg	132.0 (19.7)	133.9 (21.3)	134.8 (19.9)	130.6 (18.9)	128.9 (18.0)
Antihypertensive medication, n (%)	486 (45.8)	146 (54.7)	130 (48.0)	127 (47.4)	93 (35.0)
Diabetes, n (%)	185 (17.4)	74 (27.7)	54 (19.9)	30 (11.2)	28 (10.5)
Total cholesterol, mg/dL	189.9 (35.5)	189.6 (36.5)	188.1 (33.0)	192.3 (34.8)	189.8 (37.5)
HDL cholesterol, mg/dL	48.0 (11.6)	46.7 (10.5)	45.9 (11.1)	48.8 (12.0)	50.8 (12.3)
eGFR, mL/min per 1.73 m^2	71.2 (16.0)	70.3 (17.9)	70.2 (16.9)	72.5 (14.9)	72.1 (14.2)
History of atrial fibrillation/flutter, n (%)	67 (6.3)	22 (8.2)	11 (4.1)	18 (6.7)	17 (6.4)
History of prostate cancer, n (%)	90 (8.5)	37 (13.9)	22 (8.1)	14 (5.2)	17 (6.4)
NT-proBNP ≥300 pg/mL, n (%)	135 (12.7)	48 (18.0)	29 (10.7)	30 (11.2)	29 (10.9)
Total testosterone, ng/dL	381.4 (178.8)	178.1 (85.3)	321.3 (27.6)	416.2 (31.3)	610.0 (141.5)
cfT, ng/dL	4.80 (2.00)	2.76 (1.51)	4.59 (0.92)	5.37 (1.12)	6.48 (2.06)
Total dihydrotestosterone, ng/mL	0.44 (0.24)	0.25 (0.18)	0.39 (0.16)	0.48 (0.17)	0.65 (0.23)
cfDHT, ng/mL	0.26 (0.13)	0.18 (0.13)	0.25 (0.10)	0.28 (0.11)	0.31 (0.14)
SHBG, nmol/L	64.6 (29.5)	51.7 (30.9)	54.0 (17.6)	64.6 (19.4)	88.4 (31.3)

BMI indicates body mass index; cfDHT, calculated free dihydrotestosterone; cfT, calculated free testosterone; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and SHBG, sex hormone–binding globulin.
*Values are mean (standard deviation) or count (percent).

were no significant associations of total testosterone or cfT, total dihydrotestosterone or cfDHT, or SHBG with incident HFpEF.

DISCUSSION

Principal Findings

In this study of older men, we found that low serum concentrations of cfT were significantly associated with incident HF after full adjustment for clinical risk factors, and even potential causal intermediates. Total testosterone, total dihydrotestosterone, and SHBG, but not cfDHT, were associated with a higher risk of incident HF only in minimally adjusted models. While the risk estimates for total testosterone and total dihydrotestosterone did not change materially when each was also adjusted for SHBG levels, the associations for SHBG exhibited substantial attenuation in these models. In a secondary analysis of HF subtypes, cfT showed an inverse relationship with incident HFrEF after full adjustment, although no association was seen with incident HFpEF.

Previous Studies

Low circulating testosterone concentrations have previously been linked to increased risk of CVD[7] and mortality[24] in prospective cohorts of older men. Among middle-aged to older men, total testosterone likewise showed an inverse relationship with incident HF after

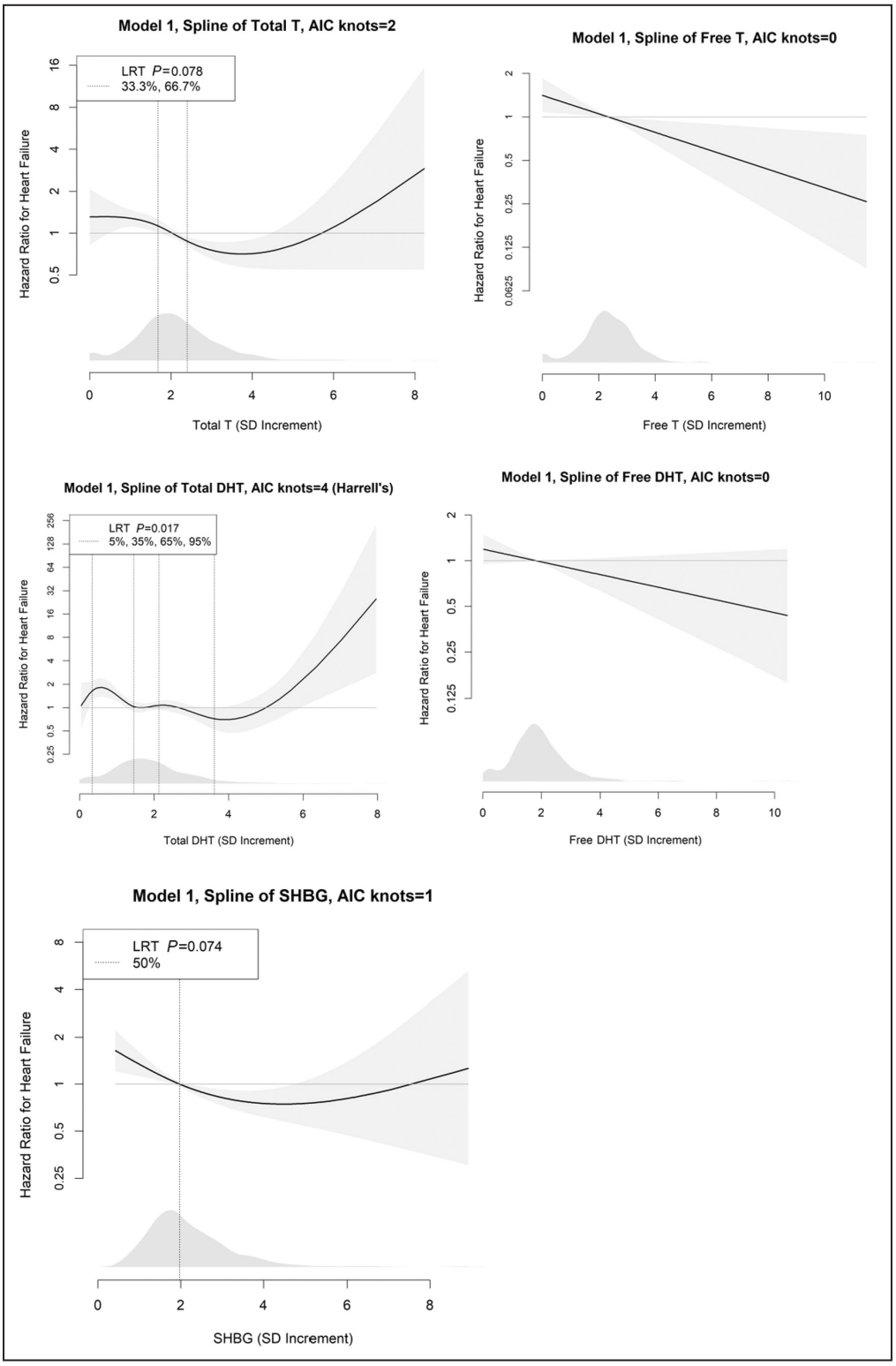

Figure 1. Graphs of predicted hazard ratios (HRs) for heart failure by hormone measures, in SD increments.

HRs are predicted from cubic spline models adjusting for age and race with knots at percentiles determined using Akaike's information criterion, selected from among models with 0 to 9 evenly spaced knots and 3 to 7 knots at Harrell's specified locations. LRT *P* value compares cubic spline model to a linear model. The density plot displayed just above the *x* axis indicates the distribution of the hormone along the spline. AIC indicates Akaike's information criterion; DHT, dihydrotestosterone; LRT, likelihood ratio test; SHBG, sex hormone–binding globulin; and T, testosterone.

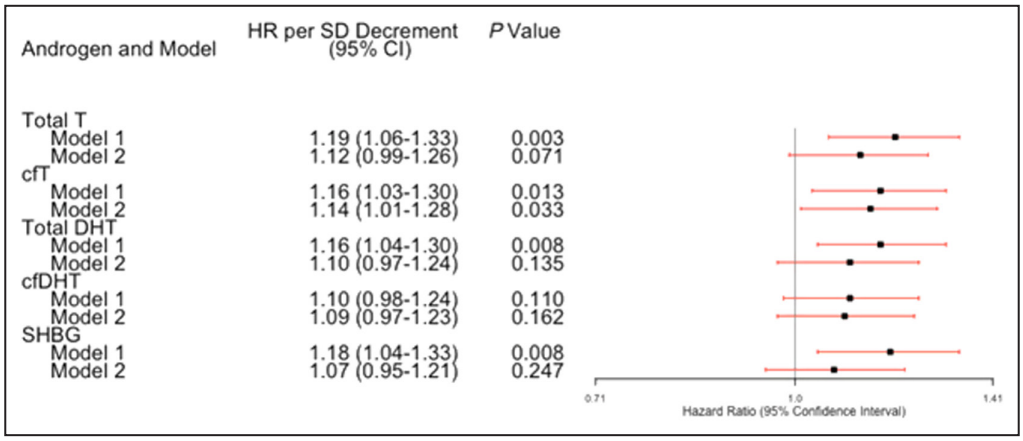

Figure 2. Associations of circulating hormone measures with incident heart failure. Model 1 adjusts for age and race.
Model 2 adjusts for age, race, body mass index, smoking, alcohol use, physical activity, systolic blood pressure, antihypertensive medication, total and high-density lipoprotein cholesterol, and estimated glomerular filtration rate. cfDHT indicates calculated free dihydrotestosterone; cfT, calculated free testosterone; DHT, dihydrotestosterone; HR, hazard ratio; SHBG, sex hormone–binding globulin; and T, testosterone.

adjustment for CVD risk factors.[8] Risk estimates for HF subtypes were of similar magnitude but nonsignificant after full adjustment. Another study of middle-aged to older men referred for coronary angiography yielded a consistent association, with cfT showing an inverse relationship with HF mortality.[25] Measurement of total testosterone in the foregoing studies, however, was mostly performed with nonstandardized immunoassays, which have limited accuracy and precision, especially at low testosterone levels.[9]

To our knowledge, the current investigation is the first to use liquid chromatography–tandem mass spectrometry determination of total testosterone and dihydrotestosterone, with Centers for Disease Control and Prevention certification for the former, to prospectively assess their relationships with incident HF in older men. The present study's inclusion of dihydrotestosterone assessment is also novel, as is its evaluation of cfT and cfDHT levels in this context. Earlier CHS analyses focused on incidence of ASCVD[13] and atrial fibrillation[14] detected a curvilinear relationship with total dihydrotestosterone and cfDHT. We found some evidence of a nonlinear relationship with incident HF only for total dihydrotestosterone. However, this occurred at extreme values with scant observations, especially at the upper range of the distribution, making the overall shape of the relationship hard to define. For all other hormone measures, we found approximately linear (inverse) relationships with incident HF, which, in contrast to the ASCVD and atrial fibrillation outcomes, proved significant for cfT. Notably, this inverse relationship of cfT was seen only for HFrEF and not for HFpEF.

Potential Explanations

While lower concentrations of nearly all androgen measures were associated with higher risk of HF after adjustment for demographic factors in our cohort, the relationship was significant for cfT only after additional adjustment for lifestyle and clinical risk factors. Because SHBG levels increase while total testosterone levels decrease with advancing age,[11] total testosterone levels are likely to be inflated among the oldest old, obscuring deficits in free testosterone in this age group. According to the free hormone hypothesis, only free testosterone is available to engage the androgen receptor, making it the preeminent measure of testosterone bioactivity.[10] Indeed, free testosterone has been shown to correlate better than total testosterone with symptoms of hypogonadism in older men.[26] Accordingly, clinical guidelines recommend determination of free testosterone concentration with either equilibrium dialysis or use of an accurate formula when total testosterone level is borderline-low or in conditions that alter SHBG levels.[9] Our findings reinforce the value of this approach for older men, supporting free testosterone as the key measure for determination of HF risk.

The relationship observed between cfT and incident HF could owe to testosterone's role as a marker of declining health or to adverse biological consequences of testosterone deficiency in older men.[27] We excluded participants with prevalent ASCVD from our analyses, and although we saw attenuation of the risk estimates for total testosterone, total dihydrotestosterone, cfDHT, and SHBG after adjustment for cardiometabolic risk factors, no meaningful attenuation

Table 2. Relationship of Hormone Measures With Incidence of HF Subtypes

Hazard ratio per SD decrement (95% CI), *P* value		
	Heart failure with preserved ejection fraction	Heart failure with reduced ejection fraction
Total testosterone		
Model 1	1.09 (0.88–1.34) *P*=0.426	1.24 (1.01–1.54) *P*=0.042
Model 2	1.01 (0.81–1.26) *P*=0.900	1.19 (0.95–1.49) *P*=0.138
Calculated free testosterone		
Model 1	1.04 (0.86–1.26) *P*=0.700	1.27 (1.02–1.56) *P*=0.030
Model 2	1.02 (0.85–1.23) *P*=0.825	1.26 (1.01–1.57) *P*=0.039
Total dihydrotestosterone		
Model 1	1.10 (0.90–1.35) *P*=0.366	1.09 (0.89–1.33) *P*=0.404
Model 2	1.04 (0.83–1.30) *P*=0.747	1.03 (0.83–1.28) *P*=0.779
Calculated free dihydrotestosterone		
Model 1	1.07 (0.87–1.31) *P*=0.548	1.05 (0.85–1.28) *P*=0.665
Model 2	1.06 (0.86–1.31) *P*=0.574	1.05 (0.85–1.29) *P*=0.680
Sex hormone–binding globulin		
Model 1	1.15 (0.92–1.45) *P*=0.214	1.14 (0.91–1.42) *P*=0.248
Model 2	1.07 (0.85–1.34) *P*=0.580	1.04 (0.83–1.30) *P*=0.735

Model 1 adjusts for age and race. Model 2 adjusts for age, race, body mass index, smoking, alcohol use, physical activity, systolic blood pressure, antihypertensive medication, total and high-density lipoprotein cholesterol, and estimated glomerular filtration rate. HF indicates heart failure.

occurred with cfT. These findings implicate the sizable influence of such risk factors on SHBG levels, but suggest a more modest contribution from these potential confounding factors to testosterone measures in these generally healthy men.[28] The cfT-HF association was substantially diminished after exclusion of participants with elevated NT-proBNP, although only a small minority of such participants met criteria for clinically unrecognized HF. Dampening of this association may be interpreted to reflect downstream consequences of low testosterone on subclinical cardiac dysfunction, although the reverse cannot be entirely excluded.

Preclinical and clinical studies support the concept that low free testosterone may play a causal role in HF onset among older men. Experimental studies in rodents have shown that testosterone repletion following orchiectomy improves calcium homeostasis in ventricular myocytes, enhancing cardiac contractility and relaxation.[29] Similarly, beneficial properties have been documented for physiologic testosterone supplementation against myocardial fibrosis and apoptosis, as have its cardioprotective effects in the setting of ischemic insults.[30,31] Moreover, testosterone undergoes conversion by aromatase to estrogen, an antioxidant hormone that protects against mitochondrial dysfunction and ischemia–reperfusion injury in experimental studies in both sexes.[31] High levels of estrogen, however, have also been documented to impair contractility and enhance fibrosis in a sex-specific manner in men.[32] Whether low testosterone concentration could adversely impact the myocardium by lowering estrogen generation is therefore unclear.

Testosterone has also been shown experimentally to have both vasodilatatory and vasoconstrictive properties[33] and both atheroprotective and proatherogenic effects.[34] The hormone's direct effects on vascular tone in the aorta and coronary arteries could influence myocardial performance through alterations in afterload or ischemia,[31] but the extent to which such mechanisms explain the relationship between low cfT and HF documented here is unclear. The impact of testosterone on CHD risk represents another area of uncertainty,[35] yet adjustment for time-varying CHD in our analyses had no effect on the observed association. Additionally, apart from its cardiovascular effects, testosterone promotes expansion of skeletal muscle mass,[36] which declines in the setting of testosterone deficiency. Because loss of skeletal muscle mass can cause exercise intolerance,[37] this could be an important contributor to the onset of HF symptoms with low testosterone levels.

It is notable that our study demonstrated an association of low cfT with HFrEF but not HFpEF. The fact that the relationship of free testosterone with HF was not affected by adjustment for CHD argues against ischemic myocardial injury as a mechanism. This points to the impact of testosterone deficiency on calcium handling and attendant impairment in myocardial contractility as a potential explanation.[30] Because aging in men is associated with mitochondrial dysfunction and cardiomyocyte apoptosis,[38]diminished anti-apoptotic effects from deficient testosterone levels could also have played a role. This is in contrast with the impact of estrogen deficiency in women, which tends to promote cardiac fibrosis and hypertrophy.[39] In this regard, our findings suggest that sex hormone deficiency could contribute to the distinct distribution of HF subtypes in men and women, wherein HFrEF predominates in men and HFpEF in women.[40] Given the secondary nature of our analysis of HF subtypes, which was possible in only ≈60% of the cohort, these findings will require replication in separate cohorts.

Despite the 3-fold higher potency of dihydrotestosterone compared with testosterone, we did not find a significant association of total dihydrotestosterone or cfDHT with HF. Previous work showed high expression of 5α-reductase in hypertrophied hearts from humans

and mice, and a reduction in left ventricular mass with administration of the 5α-reductase inhibitor finasteride in mouse models.[41] Because dihydrotestosterone is not involved in maintenance of skeletal muscle mass, this supports a substantial role for the latter in the association observed between free testosterone and incident HF.[36] In addition, because testosterone, but not dihydrotestosterone, is metabolized to produce estrogen, the lack of association for dihydrotestosterone might relate to estrogen deficiency.

Clinical Implications

The widespread use of testosterone prescriptions for questionable indications in aging men in developed countries has drawn the attention of the biomedical community and public health agencies. Lack of appropriate clinical trial data was cited as a concern, and after conduct of the Testosterone Trials showed evidence of increased coronary atherosclerosis with testosterone therapy,[42] the need for large-scale randomized trials to determine cardiovascular safety was brought to the fore.

Amid a marked rise in testosterone prescriptions for questionable indications in aging men, and concerns about adverse cardiovascular and prostate-related outcomes, the safety of testosterone replacement has become a pressing public health matter.[43] Testosterone deficiency has been recognized to be a feature of chronic HF in men, in whom it is associated with clinical severity and reduced survival.[44] In this setting, testosterone repletion has been shown to improve functional outcomes.[45] It remains undetermined whether testosterone replacement in older men with hypogonadism could lower cardiovascular and HF risk. Such repletion has been shown to increase a noncalcified coronary artery in older men with hypogonadism,[42] prompting initiation of a large-scale randomized trial (TRAVERSE [Study to Evaluate the Effect of Testosterone Replacement Therapy on the Incidence of Major Adverse Cardiovascular Events and Efficacy Measures in Hypogonadal Men]; NCT03518034) to assess the cardiovascular and prostate safety of testosterone replacement therapy. TRAVERSE focuses on atherosclerotic CVD events but does not list HF as a secondary outcome of interest. Our findings highlight the importance of this question specifically for HF—which affects ≈10% of men 80 years and older[1]—that will require evaluation in the context of the cardiovascular and urologic risks and benefits of testosterone replacement. Depending on the TRAVERSE findings, the present results suggest that this outcome of HF may merit its own focused study in men with hypogonadism.

Limitations

Limitations to our study include the moderate sample size, which precluded clear assessment of the relationship at the higher range of hormone concentrations. In the case of the nonlinear relationship observed for total dihydrotestosterone and incident HF, this was driven by a small number of values at the upper end of distribution. Additional research will be necessary to fully define the relationships with incident HF for total dihydrotestosterone and the remaining hormones, particularly at the upper extreme of their concentrations. Specimens for our study were not obtained in the fasting state, and we did not collect time of specimen collection to assess the potential effects of circadian fluctuation. Still, circadian fluctuation is blunted in older men and unlikely to be a major source of misclassification.[46] Additionally, variability in fasting status and timing of collection likely increased random variability in our hormone measures, which would tend to bias our findings toward the null hypothesis. Our longitudinal observational design is a strength but subject to possible residual confounding or reverse causation, and prevents determination of the causal basis of the documented association. We used cfT rather than directly measuring levels through equilibrium dialysis. Although this approach is less accurate, we used a validated formula deemed acceptable in recent guidelines.[9] Finally, we did not obtain concurrent estrogen measures, and cannot evaluate their corresponding impact here.

CONCLUSIONS

Among community-dwelling older men, we observed a significant prospective association between low concentration of cfT and new-onset HF. These findings support the use of cfT over total testosterone in advanced old age, and suggest the potential benefit of testosterone repletion for this highly consequential disorder for aging men. Our results provide impetus for evaluating the role of testosterone deficiency and its replacement on HF outcomes in future prospective studies.

ARTICLE INFORMATION

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Dr Kizer reports stock ownership in Abbott, Bristol–Myers Squibb, Johnson & Johnson, Medtronic, Merck, and Pfizer. The remaining authors have no disclosures to report.

Supplemental Material

Table S1

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SUPPLEMENTAL MATERIAL

Table S1. Baseline Characteristics, Both Overall and by Quartile of Calculated Free Testosterone.

Variable	Overall n=1061	Quartile 1 [0, 3.77] n=268	Quartile 2 (3.77, 4.81] n=267	Quartile 3 (4.81, 5.98] n=267	Quartile 4 (5.98, 23.8] n=268
Age, yrs	76.4 (5.1)	78.0 (5.9)	76.7 (4.8)	75.6 (4.8)	75.3 (4.1)
Black, n (%)	157 (14.8)	49 (18.3)	34 (12.7)	38 (14.2)	37 (13.8)
BMI, kg/m^2	26.7 (3.7)	27.3 (4.1)	26.8 (3.8)	26.4 (3.2)	26.2 (3.4)
Smoking, n (%)					
Never	251 (23.7)	61 (22.8)	66 (24.7)	64 (24.0)	61 (22.8)
Former	697 (65.7)	177 (66.0)	176 (65.9)	171 (64.0)	181 (67.5)
Current	113 (10.7)	30 (11.2)	25 (9.4)	32 (12.0)	26 (9.7)
Heavy Alcohol Use, n (%)	94 (8.9)	25 (9.3%)	18 (6.7)	30 (11.2)	21 (7.8)
Physical Activity, blocks/wk	51.8 (68.7)	40.4 (65.4)	51.7 (65.3)	54.4 (63.0)	61.2 (78.8)
Systolic BP, mmHg	132.0 (19.7)	134.8 (21.6)	131.1 (19.4)	133.7 (19.3)	128.3 (17.6)
Antihypertensive Medication, n (%)	486 (45.8)	126 (47.0)	132 (49.4)	115 (43.1)	118 (44.0)

Diabetes, n (%)	185 (17.4)	62 (23.1)	49 (18.4)	49 (18.4)	28 (10.4)
Total Cholesterol, mg/dL	189.9 (35.5)	189.4 (36.7)	187.5 (37.3)	189.8 (34.4)	192.7 (33.4)
HDL Cholesterol, mg/dL	48.0 (11.6)	47.5 (11.3)	47.0 (12.2)	48.7 (11.6)	48.9 (11.4)
eGFR, mL/min/1.73m^2	71.2 (16.0)	69.1 (17.6)	71.0 (16.6)	73.1 (16.0)	71.7 (13.3)
History of Atrial Fibrillation, n (%)	67 (6.3)	18 (6.7)	19 (7.1)	17 (6.4)	14 (5.2)
History of Prostate Cancer, n (%)	90 (8.5)	40 (14.9)	18 (6.7)	19 (7.1)	14 (5.2)
NT-proBNP ≥300 pg/ml, n (%)	135 (12.7)	53 (19.8)	37 (13.9)	19 (7.1)	27 (10.1)
Total T, ng/dL	381.4 (178.8)	207.5 (119.9)	354.6 (102.0)	417.4 (128.1)	545.7 (164.4)
cfT, ng/dL	4.80 (2.00)	2.47 (1.22)	4.30 (0.30)	5.35 (0.35)	7.10 (1.70)
Total DHT, ng/mL	0.44 (0.24)	0.29 (0.21)	0.43 (0.21)	0.48 (0.19)	0.57 (0.24)
cfDHT, ng/mL	0.26 (0.13)	0.16 (0.10)	0.24 (0.11)	0.29 (0.09)	0.34 (0.14)
SHBG, nmol/L	64.6 (29.5)	66.9 (34.3)	65.2 (28.2)	61.7 (27.2)	64.4 (27.3)

*Values are mean (standard deviation) or count (percent).

BMI, body mass index; cfDHT, calculated free dihydrotestosterone; cfT, calculated free testosterone; BP, blood pressure; DHT, dihydrotestosterone; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SHBG, sex hormone binding globulin; T, testosterone.