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Endogenous circulating testosterone and sex hormone-binding globulin levels and measures of myocardial structure and function: the Framingham Heart Study

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ABSTRACT

Background: The relation between endogenous testosterone concentrations and myocardial mass and function remains incompletely understood.

Objectives: To determine the cross-sectional association between endogenous hormone levels with cardiac magnetic resonance measures of myocardial mass, structure, and function in community-dwelling men across a wide age range.

Methods: A total of 720 men from the Framingham Heart Study Offspring Cohort (age range 37–82, mean = 59.6 years) who underwent cardiac magnetic resonance imaging and had hormone levels measured. Total testosterone (measured using liquid chromatography-tandem mass spectrometry), sex hormone-binding globulin (measured using an immunofluorometric assay), and calculated free testosterone levels were assessed in male participants of the Framingham Heart Study Offspring Cohort at examination 7. Cardiac magnetic resonance imaging was performed between examinations 7 and 8 (2002–2006).

Results: Age-adjusted linear regression models showed statistically significant association between total testosterone levels and left ventricular mass ($p = 0.009$), left ventricular mass index ($p = 0.006$), cardiac output ($p = 0.001$), and main pulmonary artery diameter ($p = 0.008$); the association between total testosterone and these cardiac magnetic resonance measures was weak and was not significant after adjustment for established risk factors—age, body mass index, diabetes, and hypertension. Furthermore, calculated free testosterone level was not significantly associated with any measure of myocardial mass or function. Sex hormone-binding globulin level was significantly associated with left ventricular mass ($p = 0.002$), left ventricular mass index ($p = 0.004$), cardiac output ($p = 0.003$), left ventricular ejection fraction ($p = 0.039$), and main pulmonary artery diameter ($p = 0.042$) in age-adjusted models; these associations were also rendered non-significant after adjusting for cardiovascular risk factors.

Conclusions: Neither testosterone nor sex hormone-binding globulin levels in men are associated significantly with myocardial mass and function independent of established cardiovascular risk factors.

INTRODUCTION

Case reports and uncontrolled studies have reported an increased incidence of left ventricular hypertrophy, reduced LV ejection fraction, and diastolic dysfunction among weightlifters and other athletes using androgenic-anabolic steroids (D'Andrea *et al.*, 2007; Montisci *et al.*, 2010; Far *et al.*, 2012). A cross-sectional study in power athletes who had used androgenic-anabolic steroids also showed subclinical impairment of both systolic myocardial dysfunction and diastolic myocardial dysfunction that were related to the dose and duration of androgen

use (D'Andrea *et al.*, 2007). However, a randomized controlled trial in healthy volunteers did not find significant changes in left ventricular (LV) mass or function with the administration of testosterone or nandrolone (Chung *et al.*, 2007).

Observational studies also have yielded conflicting findings on the relation between testosterone and myocardial mass and function; some studies have reported that lower testosterone is related to higher LV mass (Svartberg *et al.*, 2004; Ruige *et al.*, 2011) and higher LV ejection fraction (Ruige *et al.*, 2011), while another study in men with type 1 diabetes reported that higher levels of testosterone were associated with increased LV mass but not with higher LV ejection fraction (Hyde *et al.*, 2012). Thus, the effects of testosterone on myocardial mass and function remain poorly understood. Large epidemiologic studies examining relationship between testosterone levels with measures of myocardial mass and function have not been conducted.

Serum testosterone levels are lower in men with heart failure, and testosterone has been proposed as a therapeutic intervention to improve functional capacity in patients with heart failure (Caminiti *et al.*, 2009; Mirdamadi *et al.*, 2014a). However, initial randomized trials of testosterone in men with heart failure have reported improvements in physical performance but not in measures of cardiac function (Caminiti *et al.*, 2009; Stout *et al.*, 2012; Mirdamadi *et al.*, 2014b).

In light of the conflicting data on the relation between testosterone and myocardial mass and function, we used observational data from the Framingham Heart Study to determine whether the total and calculated free testosterone (cFT) levels are associated with measures of myocardial mass and function, including LV mass, LV ejection fraction, and cardiac output. We measured total testosterone using liquid chromatography-tandem mass spectrometry (LC-MS/MS), widely considered the reference method against which all other assays are compared, using an assay that has been certified by the Center for Disease Control's Hormone Standardization Program for Testosterone (HoST). Total testosterone (TT) levels are highly correlated with sex hormone-binding globulin (SHBG) levels and may be affected by conditions that alter the SHBG levels. Therefore, we also analyzed the association of free testosterone as well as SHBG levels with the dependent variables. We used cardiac magnetic resonance imaging (MRI) to assess standardized indices of myocardial mass and function. Cardiac MRI is a robust imaging modality for myocardial structure and functions and is being used widely in the diagnosis and follow-up of cardiovascular diseases (Chuang *et al.*, 2000, 2014; Jaffer *et al.*, 2002; Hautvast *et al.*, 2011), including heart failure and cardiomyopathies (Stokes *et al.*, 2016). The variables assessed included left ventricular mass, LV ejection fraction, and cardiac output which have proven to be robust predictors of cardiovascular mortality (Chung *et al.*, 2007; Montisci *et al.*, 2010).

SUBJECTS AND METHODS

The Framingham Heart Study (FHS) has been previously described (Cupples, 1987). The original cohort included a sample of 5209 adult male and female residents of Framingham, Massachusetts, who were recruited in 1948. In 1971, the children of the original cohort as well as their spouses were recruited to be a part of the second-generation Offspring cohort. This study used the data collected from the Offspring cohort. Sex steroid measurements were performed on serum samples obtained at examination 7 during 1998–2001. The cardiac MRI was obtained between Offspring examinations 7 and 8 (2002–2006). All participants included in this study provided written informed consent approved by the institutional review board of the Boston University Medical Center.

Cardiac magnetic resonance imaging (CMR imaging)

CMR measurements of LV mass, LV End-diastolic volume, LV ejection fraction, and cardiac output were performed on 1.5T Phillips CMR System (Gyroscan ACS NT, Philips Medical Systems, Best, The Netherlands), using a 5-element cardiac receiver coil as previously described (Chuang *et al.*, 2012). After scout images to localize the heart within the thorax, a series of contiguous slices encompassing the LV in its short-axis orientation was obtained using an electrocardiogram-gated, balanced steady-state free precision sequence (repetition time 3.2 ms, echo time 1.6 ms, flip angle 60°). Slice thickness was 10 mm (no gap) with $1.9 \times 1.6\text{-mm}^2$ in-plane spatial resolution. One slice was acquired per end-tidal breath hold; no acceleration technique was used. Images were analyzed by a single, experienced, blinded reviewer using a commercial workstation (EasyVision 4.0; Philips Medical Systems). End-systolic phase was

Table 1 Characteristics of participants included in the study

Variables	Overall (N = 720)
Age at Offspring examination 7 (years)	59.6 (9.1)
BMI (kg/m^2)	28.5 (4.2)
Smokes cigarettes	68 (9.5%)
Systolic blood pressure (mmHg)	126.3 (16.2)
Diastolic blood pressure (mmHg)	76.3 (9.3)
Hypertension	305 (42.4%)
Heart rate (BPM)	63.4 (11.8)
Fasting glucose (mg/dL)	105.5 (24.9)
Diabetes status	69 (9.6%)
Prevalent CVD	71 (9.9%)
HDL cholesterol (mg/dL)	45.8 (12.5)
LDL cholesterol (mg/dL)	122.0 (29.6)
Total cholesterol (mg/dL)	194.5 (33.1)
Triglyceride (mg/dL)	139.6 (99.3)
Total testosterone (ng/dL)	598.1 (229.5)
	572.2 (430.0, 731.5)
Free testosterone (pg/mL)	91.1 (34.9)
	85.0 (69.0, 106.0)
SHBG (nmol/L)	56.8 (25.8)
	52.0 (39.4, 69.9)
LV mass	127.8 (25.0)
	124.3 (110.8, 143.0)
LV mass index	28.2 (5.5)
	27.3 (24.4, 31.1)
LV end-diastolic volume	144.8 (27.9)
	142.9 (125.7, 161.2)
LV ejection fraction	65.9 (6.7)
	66.0 (61.7, 70.0)
LV stroke volume (mL)	94.7 (17.4)
	93.8 (83.4, 105.8)
Cardiac output (L/min)	5.9 (1.2)
	5.8 (5.1, 6.6)
Short-axis LV septal wall thickness (mm)	8.5 (1.3)
	8.4 (7.6, 9.3)
Main pulmonary artery diameter (mm)	23.8 (2.9)
	23.6 (21.8, 25.5)

Values expressed as mean and standard deviation or number and percent. For hormone and MRI, data values for medians and interquartile ranges are also shown. BMI: body mass index; CVD: cardiovascular disease; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SHBG: sex hormone-binding globulin; LV: left ventricular.

determined as the minimal cross-sectional area of a mid-ventricular slice. The time delay from the QRS complex phase was analyzed for each contiguous slice, and endocardial borders were segmented. End-diastolic volume (EDV) and end-systolic volume (ESV) were computed by summation of disks (i.e., modified Simpson's rule) to derive the LVEF ([EDV − ESV]/EDV). Cardiac output (L/min) calculated as (EDV-ESV) multiplied by heart rate. Inter-rater reliability correlation coefficients were as follows: for EDV = 0.95 and for ESV = 0.92. Intra-observer coefficient of variation for EDV was 2.6% and for ESV was 3.5%. Inter-observer coefficient of variation for EDV was 3.5% and ESV was 4.8% (Chuang *et al.*, 2000).

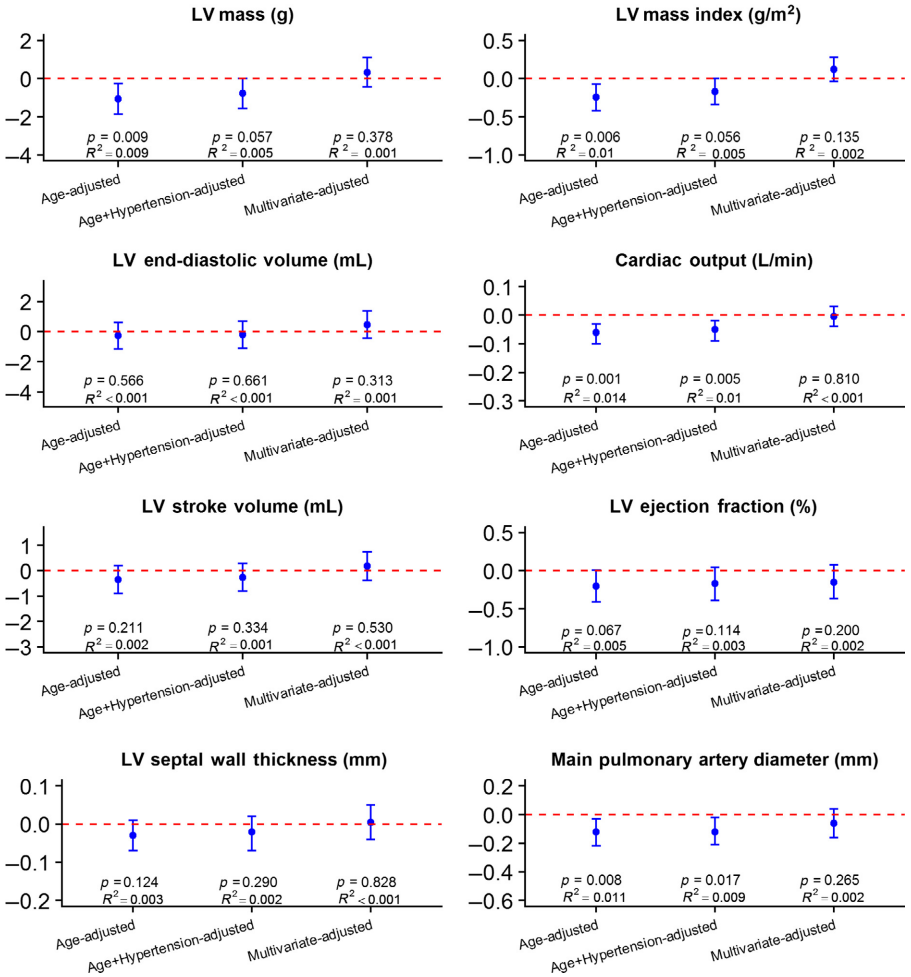
Hormone measurement

Blood samples were drawn in the supine position in the early morning after an overnight fast. Sera were aliquoted and immediately stored at −70 °C, remaining frozen until the time of the assay. Serum total testosterone levels were measured by LC-MS/MS, in a laboratory certified by the CDC's HoST program, as previously described (Bhasin *et al.*, 2011a). The functional sensitivity of the total T assay was 0.07 nmol/L, and the interassay coefficient of variation was 15.8% at 0.42 nmol/L, 10.6% at 0.82 nmol/L, 7.9%, at 1.7 nmol/L, 7.7% at 8.4 nmol/L, 4.4% at 18.5 nmol/L, and 3.3% at 35.3 nmol/L, respectively. Quality control samples, sent by the CDC's HoST Program, were tested every 3 months; the bias in quality control samples with testosterone concentrations in the 3.5–35 nmol/L range was consistently less than 6.2%. The limit of quantitation for the total testosterone level was 1 ng/dL. SHBG was measured using a two-site directed immunofluorometric assay that had a sensitivity of 0.5 nM/L (Delfia, Wallac Oy, Turku, Finland). Free testosterone (cFT) concentrations were calculated from total testosterone and SHBG concentrations, using a law of mass action equation (Mazer, 2009).

Cardiovascular risk factors

Standard anthropometric and risk factor measurements were obtained as previously described (Kannel *et al.*, 1979). Age was determined at the date of the participant examination visit (at which blood was obtained). Body mass index (BMI) was computed from standardized measures of height and weight obtained during clinical study visits. Information regarding medication usage was collected. Prevalent cardiovascular disease (CVD) was defined as history of coronary heart disease, congestive heart failure, stroke, or transient ischemic attack. Diabetes was defined as a fasting glucose >126 mg/dL, a random glucose

Figure 1 Age-adjusted and multivariate-adjusted effects of total testosterone levels on cardiac outcomes. Effects and 95% CIs are expressed per 100 ng/dL units of total testosterone. *p*-Values for total testosterone effects on cardiac outcomes are derived from 3 models: adjusted to age only; adjusted to age and hypertension; and adjusted to age, BMI, hypertension, and diabetes status (multivariate-adjusted model). Partial R^2 value for total testosterone extracted from linear regression model.



>200 mg/dL, or taking antidiabetic medications. Hypertension was diagnosed by the presence of a systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or reported use of antihypertensive medication. Current smokers reported smoking at least one cigarette per day over the preceding year.

Analytic sample

A total of 835 men in the Framingham Offspring cohort had CMR imaging performed between examination cycles 7 and 8, 66 of these men had prevalent myocardial infractions, 47 had hormone values missing, 1 was on testosterone treatment, and 1 was on prostate cancer treatment. Analyses were thus carried out on 720 participants from the Offspring cohort that were free of prevalent MI at the time of the FHS examination, had hormone data available, and were not on hormone therapy.

Statistical analysis

Baseline characteristics of the participants are expressed as mean and standard deviations for continuous variables and the number and proportions of subjects for categorical variables. Additionally, medians and interquartile ranges were provided for cardiac measures. Unrestricted cubic splines were constructed to investigate potential non-linearity in examined relationships (Hastie *et al.*, 2001). Pearson correlation coefficients were calculated for our variables of interest, and variance inflation factor was derived to assess multicollinearity among covariates. Age-adjusted and multivariable-adjusted linear regression models were performed to determine the association and its magnitude, between cardiac mass; structure; function metrics; and TT, cFT, and SHBG levels. For each cardiac outcome, 3 models were considered: age-adjusted, age- and hypertension-adjusted and multivariate-adjusted model with age, BMI, hypertension, and diabetes status included as covariates. Estimates of age-adjusted and multivariate-adjusted effects of hormone levels, with corresponding 95% CIs, were provided per 100 ng/dL of TT, per 10 pg/mL of calculated FT and per 10 nmol/L of SHBG level. Relative strength of association between hormone data and outcomes was assessed by R-squared and partial R-squared metrics. Robustness of our findings was further investigated through sensitivity analyses performed on healthy cohort (participants free of CVD, diabetes, hypertension with BMI less than 30 kg/m^2).

All tests use two-sided 0.05 alpha levels. Analyses were performed using SAS v.9.3 (SAS Institute, Cary, NC) and R software version 3.2.5.

RESULTS

Characteristics of study sample

The participants were on average 60 years old and overweight (mean (SD): BMI=28.5 (4.2) kg/m^2); 42% were hypertensive,

Table 2 Estimated differences (and 95% CIs) in cardiac outcomes per change in sex steroid levels

Outcome	Sex steroids		
	Total testosterone	Free testosterone	SHBG
LV mass			
Age-adjusted	−1.06 (−1.86, −0.26)	−1.57 (−7.02, 3.88)	−11.37 (−18.67, −4.06)
Age- and hypertension-adjusted	−0.77 (−1.56, 0.02)	−0.35 (−5.72, 5.02)	−9.32 (−16.52, −2.11)
Multivariate-adjusted	0.34 (−0.42, 1.11)	3.64 (−1.40, 8.67)	−1.75 (−8.62, 5.12)
LV mass index			
Age-adjusted	−0.24 (−0.42, −0.07)	−0.42 (−1.62, 0.77)	−2.36 (−3.96, −0.77)
Age- and hypertension-adjusted	−0.17 (−0.34, 0.004)	−0.10 (−1.27, 1.06)	−1.87 (−3.43, −0.30)
Multivariate-adjusted	0.12 (−0.04, 0.28)	0.94 (−0.12, 1.99)	0.11 (−1.33, 1.55)
LV end-diastolic volume			
Age-adjusted	−0.26 (−1.14, 0.62)	−1.19 (−7.18, 4.80)	−0.81 (−8.98, 7.26)
Age- and hypertension-adjusted	−0.20 (−1.09, 0.69)	−0.99 (−7.01, 5.03)	−0.29 (−8.41, 7.83)
Multivariate-adjusted	0.47 (−0.44, 1.38)	1.40 (−4.59, 7.40)	4.24 (−3.92, 12.40)
Cardiac output			
Age-adjusted	−0.06 (−0.10, −0.03)	−0.24 (−0.50, 0.01)	−0.51 (−0.85, −0.17)
Age- and hypertension-adjusted	−0.05 (−0.09, −0.02)	−0.21 (−0.46, 0.05)	−0.44 (−0.78, −0.11)
Multivariate-adjusted	−0.004 (−0.04, 0.03)	−0.02 (−0.26, 0.22)	−0.11 (−0.44, 0.21)
LV stroke volume			
Age-adjusted	−0.35 (−0.90, 0.20)	−1.36 (−5.10, 2.38)	−3.02 (−8.06, 2.02)
Age- and hypertension-adjusted	−0.27 (−0.83, 0.28)	−1.09 (−4.83, 2.65)	−2.33 (−7.38, 2.71)
Multivariate-adjusted	0.18 (−0.38, 0.74)	0.61 (−3.09, 4.31)	0.73 (−4.31, 5.77)
LV ejection fraction			
Age-adjusted	−0.20 (−0.41, 0.01)	−0.71 (−2.16, 0.73)	−2.05 (−4.00, −0.10)
Age- and hypertension-adjusted	−0.17 (−0.39, 0.04)	−0.63 (−2.08, 0.82)	−1.80 (−3.74, 0.15)
Multivariate-adjusted	−0.15 (−0.37, 0.08)	−0.48 (−1.95, 0.98)	−1.60 (−3.59, 0.40)
LV septal wall thickness			
Age-adjusted	−0.03 (−0.07, 0.01)	−0.02 (−0.31, 0.27)	−0.35 (−0.73, 0.04)
Age- and hypertension-adjusted	−0.02 (−0.07, 0.02)	0.03 (−0.26, 0.31)	−0.29 (−0.67, 0.10)
Multivariate-adjusted	0.005 (−0.04, 0.05)	0.12 (−0.16, 0.41)	−0.10 (−0.49, 0.29)
Main pulmonary artery diameter			
Age-adjusted	−0.13 (−0.22, −0.03)	−0.54 (−1.19, 0.11)	−0.91 (−1.79, −0.03)
Age- and hypertension-adjusted	−0.11 (−0.21, −0.02)	−0.49 (−1.14, 0.16)	−0.83 (−1.71, 0.05)
Multivariate-adjusted	−0.06 (−0.16, 0.04)	−0.25 (−0.90, 0.40)	−0.39 (−1.28, 0.49)

Estimated differences and 95% CIs are extracted from linear regression model and expressed per 100 ng/dL units of total testosterone, per 10 pg/dL units of free testosterone, and per 10 nmol/L units of SHBG.

10% had diabetes mellitus, 10% had cardiovascular disease, and 10% were current smokers (Table 1). Mean total and free testosterone, and SHBG levels were in the normal male range. Similarly, the median LV mass, LV end-diastolic volume, LV ejection fraction, LV stroke volume, and cardiac output were normal for adult men (Table 1).

Association of total and free testosterone and SHBG levels with cardiac measures

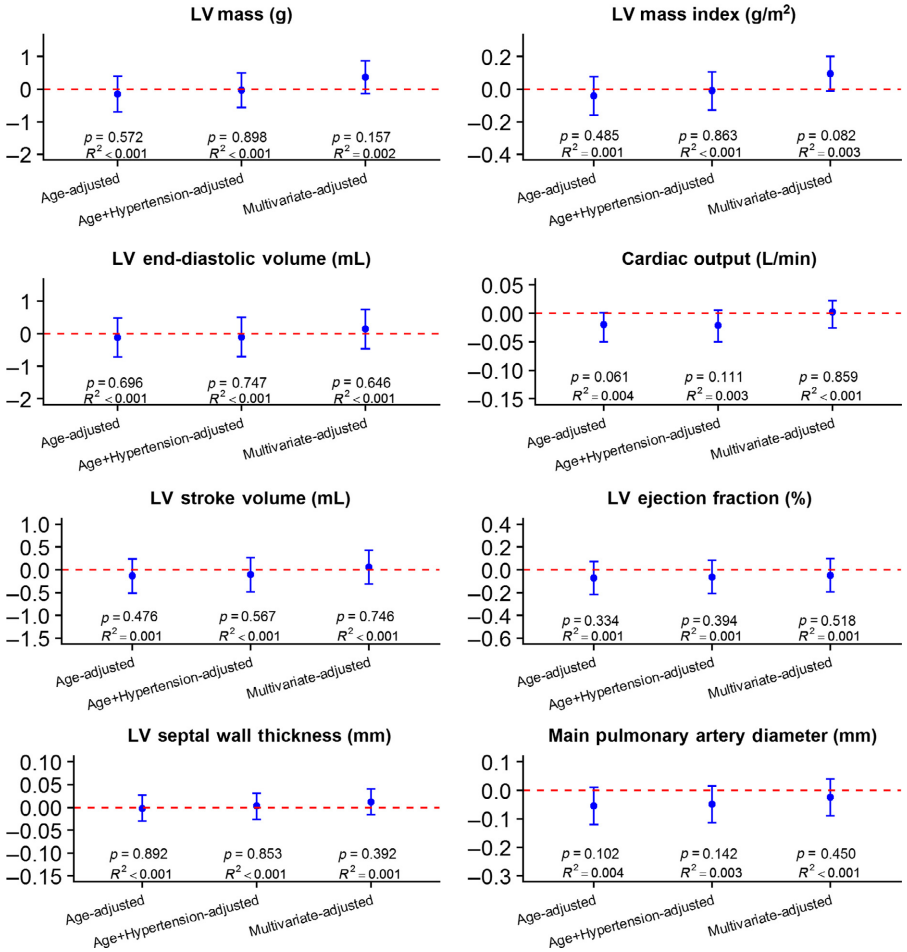
The analyses of unrestricted cubic splines did not manifest non-linear associations between hormone levels and any cardiac measures. Total and free testosterone, and SHBG levels were negatively associated with BMI (Table S1) (all $p < 0.001$). Total testosterone levels were negatively associated with prevalent diabetes and hypertension status ($p = 0.003$ and $p < 0.001$, respectively). There was no multicollinearity detected among covariates as variance inflation factors were low (below 1.3) in all considered regressions.

In age-adjusted models, total testosterone levels were negatively associated with LV mass, LV mass index, and cardiac output (Fig. 1). However, the associations were weak (Table 2). However, the age-adjusted models for the association of free testosterone levels and cardiac measures were not statistically significant for any analyzed measure (Fig. 2, Table 2). Because total testosterone levels are highly correlated with SHBG levels, we considered the possibility that the association of total testosterone with these cardiac measures was being driven by the association of SHBG with these cardiac measures, as has been observed for diabetes and metabolic syndrome (Ding *et al.*, 2009). Indeed, SHBG levels were significantly negatively associated with LV mass, LV mass index, cardiac output, and LV ejection fraction (Fig. 3). These relationships were of small magnitude (Table 2).

Cardiovascular risk factor adjustment

We performed age and hypertension-adjusted and multivariate-adjusted models with inclusion of standard cardiovascular risk factors (age, BMI, diabetes, and hypertension). Multivariate models, with age, hypertension, diabetes, and BMI adjustment did not show statistically significant association in any of the analyzed regressions (Figs 1–3). In multivariate linear regression with age, BMI, hypertension, and diabetes adjustments, 100 ng/dL difference in TT was associated with 0.34 g (95% CIs: −0.42, 1.11) and 0.12 g/m^2 (95% CIs: −0.04, 0.28) estimated difference in LV mass and LV mass index, respectively. Similarly, a 10 nmol/L difference in SHBG was associated with −1.75 g (95%

Figure 2 Age-adjusted and multivariate-adjusted effects of free testosterone levels on cardiac outcomes. Effects and 95% CIs are expressed per 10 pg/mL units of free testosterone. *p*-Values for free testosterone effects on cardiac outcomes are derived from 3 models: adjusted to age only; adjusted to age and hypertension; and adjusted to age, BMI, hypertension, and diabetes status (multivariate-adjusted model). Partial R^2 value for free testosterone extracted from linear regression model.



CIs: −8.62, 5.12) and 0.11 g/m^2 (95% CIs: −1.33, 1.55) difference in LV mass and LV index, respectively. After cardiovascular risk factor adjustment, the associations between total testosterone, SHBG, and cardiac output were not significant (Table 2). Free testosterone was not significantly associated with any measure of myocardial mass or function in CV risk-adjusted models (Fig. 2, Table 2).

The strength of association between hormone levels and measures of myocardial mass and function assessed by R^2 and partial R^2 metrics confirmed the weak associations, supporting the findings of the multivariate analyses (partial R^2 for hormone levels were <1.0%; Figs 1–3).

Sensitivity analyses

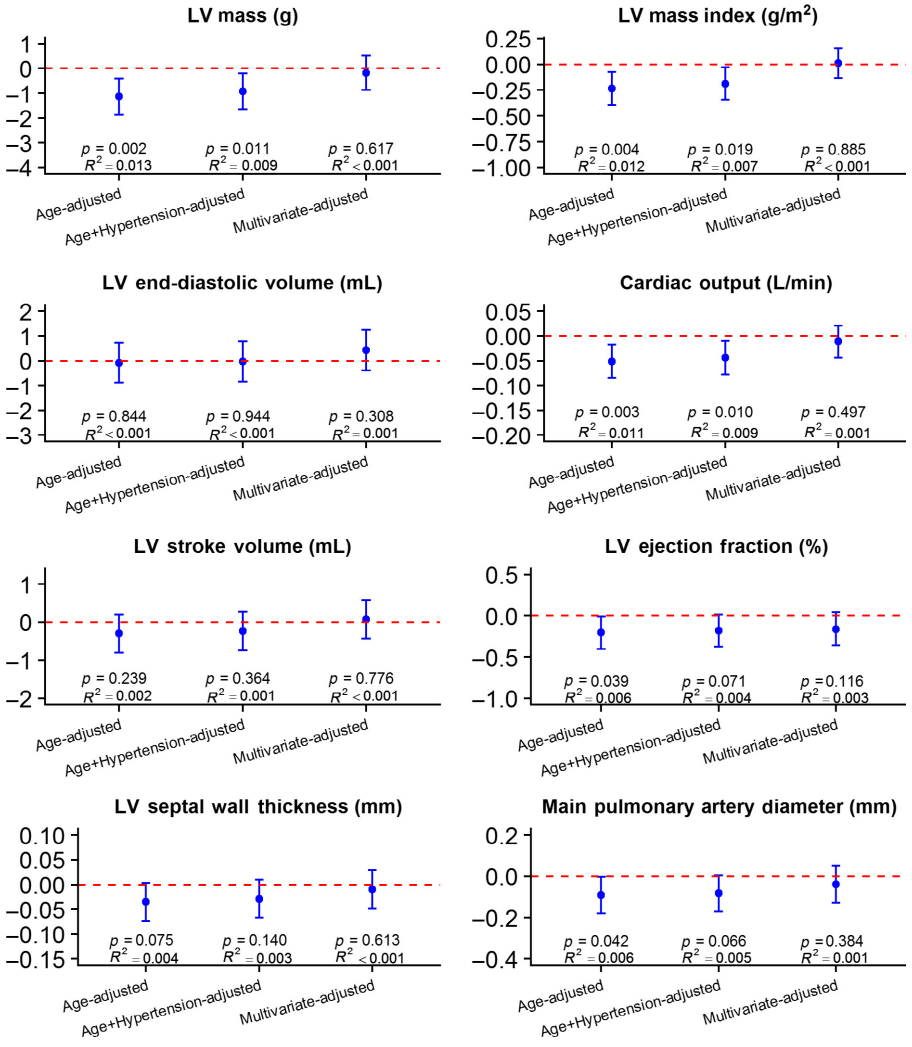
The analyses of age- and hypertension-adjusted regressions for measures of myocardial mass and function on sex steroid levels showed similar results to multivariable-adjusted relationships (Table 2, Figs 1–3). Sensitivity analyses for age-adjusted regressions performed on healthy participants with BMI < 30 kg/m^2, and without cardiovascular event, diabetes, or hypertension, demonstrated significant and negative relationship between SHBG and LV mass (p = 0.011), cardiac output (p = 0.010), and LV ejection fraction (p = 0.011); however, all linear associations for total and free testosterone were not statistically significant (Table S2).

DISCUSSION

These cross-sectional analyses of data from men in the Offspring cohort of the FHS found no significant association between total or free testosterone concentrations and measures of myocardial mass and function, specifically LV mass, LV mass index, cardiac output, LV stroke volume, or LV ejection fraction, independent of cardiovascular risk factors. Although there were several statistically significant associations between total testosterone concentrations and measures of myocardial mass and function, these associations were weak in age-adjusted models and were no longer significant after adjusting for important cardiovascular risk factors, such as age, BMI, history of CVD, hypertension, and diabetes status. These findings lead us to conclude that neither total nor free testosterone levels are associated with myocardial mass or function, independently of well-established cardiovascular risk factors.

Another novel finding of our analyses is the observed association of circulating SHBG levels with LV mass, LV mass index,

Figure 3 Age-adjusted and multivariate-adjusted effects of SHBG levels on cardiac outcomes. Effects and 95% CIs are expressed per 10 nmol/L units of SHBG. *p*-values for SHBG effects on cardiac outcomes are derived from 3 models: adjusted to age only; adjusted to age and hypertension; and adjusted to age, BMI, hypertension, and diabetes status (multivariate-adjusted model). Partial R^2 value for SHBG extracted from linear regression model.



cardiac output, and LV ejection fraction. Although novel and interesting, these associations between SHBG and measures of myocardial mass and function were weak, explained less than 1% of the variance in these variables, and therefore likely to be of limited physiologic significance. Because total testosterone levels are highly correlated with SHBG levels, the observed association of total but not free testosterone levels with LV mass, LV mass index, cardiac output, and LV ejection fraction was likely driven by the collinearity between total testosterone and SHBG levels. These findings add to the growing body of data that suggest that SHBG may be an important marker of cardiometabolic risk (Hair *et al.*, 2010; Bhasin *et al.*, 2011b).

The analyses performed in this study have many strengths but also some limitations. Unlike previous studies, total testosterone levels were measured using LC-MS/MS, the method with the highest accuracy and sensitivity, in a laboratory certified by the CDC's HoST program. Myocardial mass and function were measured using cardiac magnetic resonance imaging, widely considered an excellent measure of cardiac structure and function (Francois, 2015; Juan *et al.*, 2015; Pattanayak & Bleumke, 2015). The population studied included men over a wide age range (37–82). The FHS uses robust procedures for recording and ascertaining cardiovascular outcomes. However, the FHS cohort consists mostly of persons of Caucasian origin, which might hinder the generalizability of these findings. The analyses were cross-sectional. Epidemiologic studies can only demonstrate associations, but cannot establish causality. In fact, reverse causality cannot be excluded.

The effects of testosterone on myocardial mass and function in animal models have been conflicting and have varied with the animal species and strain. Testosterone has been reported to induce myocardial hypertrophy in some preclinical models but not in others. Our findings are consistent with the results of small randomized trials which have shown no improvements in measures of cardiac function. Testosterone treatment is being explored as a therapeutic intervention to improve physical performance in patients with heart failure. Low circulating testosterone levels have been associated with reduced exercise capacity and poor outcomes in patients with heart failure. A few small clinical trials of the effects of testosterone replacement in men with heart failure have been conducted (Malkin *et al.*, 2006; Caminiti *et al.*, 2009) with variable results; a meta-analysis of 4 clinical trials found that testosterone treatment was associated with a significantly greater improvement in performance in the 6-min walk test, incremental shuttle test, and peak oxygen consumption, compared with placebo (Toma *et al.*, 2012). Differences in the findings of epidemiologic studies and randomized trials may reflect the variable effects of physiologic concentrations of endogenous pulsatile hormone vs. exogenously administered hormone, and the different at-risk time durations.

Observational studies of athletes and non-athlete weightlifters, who had used one or more anabolic androgenic steroids, have reported evidence of left ventricular hypertrophy and systolic as well as diastolic dysfunction among lifetime users of these compounds compared to nonusers (D'Andrea *et al.*, 2007; Baggish *et al.*, 2017). The findings of systolic and diastolic dysfunctions in small case reports of powerlifters and recreational bodybuilders are difficult to extrapolate to healthy community-dwelling men because in weightlifters, the effects of androgens cannot be easily separated from those of intense resistance exercise training (Pope *et al.*, 2014). Also, the effects of highly supraphysiologic doses of androgens, often used in combination with other drugs in the setting of high-risk behaviors, may differ from those of physiologic testosterone concentrations in healthy men (Pope *et al.*, 2014).

CONCLUSIONS

In summary, our analyses do not reveal a significant and clinically meaningful association of testosterone levels with measures of myocardial mass or function. Further prospective studies are needed to elucidate the role of androgen in the regulation of myocardial mass and function in men, if any. However, the results of our analyses do not support an important role for circulating testosterone levels in regulation of myocardial mass and function in adult men.

FUNDING

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DISCLOSURE

Dr. Basaria has previously received grant support from Abbott Pharmaceuticals for an investigator-initiated clinical trial unrelated to this study and has previously consulted for Abbvie, Eli Lilly, Inc, and Regeneron Pharmaceuticals. Dr. Bhasin reports receiving research grant from NIA, NINR, NICHD-NCMRR, Alivegen, AbbVie, and MIB, and Transition Therapeutics and consultation fees from AbbVie and OPKO.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Pearson correlation coefficients with corresponding *p*-values for association between sex steroids and cardiovascular risk factors

Table S2 Estimated differences (and 95% CIs) in cardiac outcomes per difference in sex steroid levels in healthy cohort

