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Association of total and free testosterone with cardiovascular disease in a nationally representative sample of white, black, and Mexican American men

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Abstract

Associations of total testosterone (T) and calculated free T with cardiovascular disease (CVD) remain poorly understood. Particularly how these associations vary according to race and ethnicity in a nationally representative sample of men. Data included 7058 men (≥20 years) from NHANES. CVD was defined as any reported diagnosis of heart failure (HF), coronary artery disease

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AUTHOR CONTRIBUTIONS

DSL, ST, and KKT contributed to the study design, interpretation of the data, writing and critical discussion of the paper draft. ST and SG contributed to the statistical analysis of the study. All other authors contributed to the interpretation, discussion and editing of the paper draft.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

The protocols for the conduct of the NHANES were approved by the Institutional Review Board. Informed consent was obtained from all participants.

INFORMED CONSENT

We conducted a secondary data analysis using data from NHANES. NHANES obtained informed consents from participants that also included consent for publication.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41443-022-00660-7.

(CAD), myocardial infarction (MI), and stroke. Total T (ng/mL) was obtained among males who participated in the morning examination. Weighted multivariable-adjusted logistic regression models were conducted. We found associations of low T (OR = 1.57, 95% CI = 1.17–2.11), low calculated free T (OR = 1.53, 95% CI = 1.10–2.17), total T (Q_1 vs Q_5), and calculated free T (Q_1 vs Q_5) with CVD after adjusting for estradiol and SHBG. In disease specific analysis, low T increased prevalence of MI (OR = 1.72, 95% CI = 1.08–2.75) and HF (OR = 1.74, 95% CI = 1.08–2.82), but a continuous increment of total T reduced the prevalence of CAD. Similar inverse associations were identified among White and Mexican Americans, but not Blacks (OR = 0.93, 95% CI = 0.49–1.76). Low levels of T and calculated free T were associated with an increased prevalence of overall CVD and among White and Mexican Americans. Associations remained in the same direction with specific CVD outcomes in the overall population.

INTRODUCTION

Testosterone (T) levels have been previously suggested to be associated with premature death, muscle strength, and body fatness [1–3]. However, the effects of T deficiency or low levels of T (total T ≤ 300 ng/dL or 3.0 ng/mL) [4] on cardiovascular disease (CVD) remained inconsistent and controversial [5]. In parallel, this inconsistency and controversy has been reported with low levels of calculated free T [6, 7].

Studies have suggested that as many as 38.7% of men in the United States (US) over 45 years old demonstrate low T or T deficiency [8–10] with close to 2.4 million men (aged 40–69 years) with T deficiency [11]. However, a greater concern remains that it has been projected that by 2025 ~6.5 million US men (aged 30–80 years) will develop T deficiency, partly due to the increasing rate of aging population and the obesity epidemic [2, 9, 12]. A recent report indicates that 25% of men aged >65 years have low total T, but at least 50% of them have low levels when using free T as the diagnostic criterion suggesting that free T can be a better test for T deficiency/hypogonadism diagnosis [13]. Yet, this contention remains debatable [14].

Previous studies have provided valuable insight, but they have had small samples of minority populations with limited generalizability to non-Hispanic (NH)-Black and Mexican American men [15, 16]. The latter homogenous group constitutes more than 60% of the Hispanic population in the US [17]. Previous studies have demonstrated racial and ethnic differences (NH-White, NH-Black and Mexican Americans) with total T levels among adult and adolescent men [18], and in association with CVD [19].

Therefore, the objectives of this investigation is to determine the association of total serum T and calculated free T with CVD, and its specific disease outcomes (myocardial infarction [MI], heart failure [HF], coronary heart disease [CAD]), and to assess whether these associations vary among a US nationally representative sample of NH-White, NH-Black, and Mexican American men in the NHANES waves full sample [1988–1991, 1999–2004, and 2011–2016] and subset sample [1988–1991, 1999–2004, and 2013–2016]). We hypothesize that these associations will vary by race and ethnicity.

METHODS

Study population

The National Health and Nutrition Examination Survey (NHANES) is a program from the National Center for Health Statistics (NCHS)- Centers for Disease Control and Prevention (CDC) to investigate the health of adults and children in the US [20]. NHANES is a prevalent study that uses a multistage, stratified and clustered probability sampling strategy in which Hispanics (Mexican Americans), NH-Blacks, and the elderly are oversampled to ensure adequate sample size and to represent the total US civilian, non-institutionalized population [21]. Information about the survey design, data collection and methodology is available on the NHANES website (https://wwwn.cdc.gov/nchs/nhanes/Default.aspx. Accessed Jan. 2020).

This investigation included men in the 1988–1991 (Phase 1), 1999–2004, and 2011–2016 NHANES cycles. Sex steroid hormones were measured from stored surplus serum samples by the study investigators in 7058 males aged ≥20 y. These participants were stratified in a random sample in the morning examination sessions of each cycle to reduce extraneous variation due to diurnal production of hormones.

Participants with prostate cancer history were excluded because their treatments may affect sex steroid hormone. Exclusion criteria included men younger than 20 y, covariates' missing information, missing sex hormone measurements and having extreme hormone measurements leaving a final full sample of 7058 males. NHANES 2011–2012 wave did not measure estradiol and SHBG, and therefore a subset sample of 1988–1991, 1999–2004, and 2013–2016 waves was developed to adjust for estradiol and SHBG ($n = 5139$).

Assessment of testosterone, estradiol and SHBG

Information on the blood draw, process, storage and shipping methods was published elsewhere [21]. NHANES 1988–1991 and 1999–2004 measured total T, estradiol, and SHBG using the electrochemiluminescence immunoassays on the 2010 Elecsys system (Roche Diagnostics, Laval, QC, Canada; and Roche Diagnostics, Indianapolis, IN, USA). The lower limits of detection of the assays were 3 nmol/L for SHBG, 5 pg/mL for estradiol, and 2 ng/dL for T. Duplicates ($n = 21$) were assayed for quality control purposes: coefficients of variation were 4.8% for testosterone, 21.4% for estradiol, and 5.6% for SHBG. NHANES 2011–2016 measured total T and estradiol with LC-MS/MS and isolated from 100 μL serum by 2 serial liquid–liquid extraction steps and quantified with [^{13}C] stable isotope–labeled T as the internal standard. The lower limit of detection was 0.3 ng/dL. Sex hormone binding globulin (SHBG) was measured based on the reaction of SHBG with immunoantibodies and chemoluminescence measurements of the reaction products and subjecting to a magnetic field.

Total T below or equal to 3.0 ng/mL was defined as low T or T deficiency [4]. Total T was also categorized (quintiles [Q]) to compare the prevalence of CVD between Q_5 vs Q_1 of total T under the hypothesis that high levels of T is a potential risk factor for CVD. Calculated free T was obtained by published formulas with information for total T, estradiol, SHBG, and serum albumin collected in NHANES [22, 23]. Free T below or equal to ≤0.065 ng/mL was considered low per expert opinion noted in the American Urological Association White Paper- Paduch et al [24]. Calculated free T was also categorized (quintiles) to compare the prevalence of of CVD between Q_5 vs Q_1 of calculate free T.

Assessment of cardiovascular disease (CVD)

In this study, we defined CVD as any reported diagnosis of HF, coronary artery disease (CAD), MI, and stroke. NHANES participants were asked the following structure questions: “Has a doctor or other health professional ever told you that you had congestive HF?,” “Has a doctor or other health professional ever told you that you had CAD?,” “Has a doctor or other health professional ever told you that you a heart attack (or MI)?,” or “Has a doctor or other health professional ever told you that you a stroke?.” Participants who answered “yes” to any of these questions were included in the positive status of CVD.

Assessment of covariates

Age, cigarette smoking, race/ethnicity, alcohol consumption, education, diabetes and physical activity during the past 30 days were self-reported during the NHANES interviews. Glucose was defined in NHANES using the glucose hexokinase method with a Hitachi Model 704 multichannel analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). Body mass index (BMI) was measured as weight in kilograms divided by height in meters squared. Overall obesity was defined by BMI ≥ 30 kg/m^2. Individuals were classified as having diabetes if their fasting plasma glucose levels were ≥126 mg/dl, or if they responded positively to questions about medication treatment or being “told by a doctor you have diabetes or sugar diabetes. Three readings of systolic and diastolic blood pressure were obtained from participants who attended the mobile examination center. We used the average of those three measurements (≥140/90 mmHg). We also considered the current use of antihypertensive medication treatment or being “told by a doctor you have hypertension” as an indication of high blood pressure (hypertension). Serum total cholesterol was measured enzymatically [25], and serum lipid measurement was performed according to the criteria of CDC’s Lipid Standardization Program [26]. Details related to the laboratory procedures have been published previously [21]. Code availability upon request to corresponding author.

Statistical analysis

Sampling weights were applied to account for selection probabilities, oversampling, non-response, and differences between the sample and the total US population. Geometric means and 95% confidence intervals (CI’s) for total T, calculated free T, estradiol and SHBG concentrations were estimated in the full and subset samples. For this analysis, total T concentrations were transformed using natural logarithm because they were right skewed. The information on the definition has been published previously [21]. In brief, for descriptive analysis, we compared the distribution of lifestyle and sociodemographic factors by full and subset samples using t-test statistic for means from continuous variables and chi-squared for categorical factors (Table 1 and Supplemental Table 1).

We used weighted logistic regression models to estimate multivariable-adjusted odd ratios and 95% CI’s for prevalent CVD and specific outcomes (HF, CAD and MI) associated independently with total T and calculated free T. Weighted multivariable adjusted analyses were performed in 2 models, namely, full sample [1988–1991, 1999–2004, and 2011–2016] and subset sample [1988–1991, 1999–2004, and 2013–2016]. The full sample models were adjusted for race and ethnicity, age, smoking status, education, history of hypertension, physical activity, alcohol consumption, BMI, diabetes, and total cholesterol. In parallel, the subset sample models were adjusted for same risk factors plus estradiol and SHBG [27], which were not included in NHANES 2011–2012. In order to test for a linear trend across categories of total T and calculated free T, we modeled categories of total and calculated free T as continuous variables using the median for each category.

Stratified and weighted multivariable adjusted analyses were conducted by race and ethnicity (NH-White, NH-Black, and Mexican American) because this factor has been observed to modify T levels [18]. All p values were two-sided; alpha = 0.05 was considered the cut-off for statistical significance. Multiplicative interactions terms were incorporated into the models and tested using the Wald test. All statistical analyses were performed using SAS (SAS Institute v.9.4, Cary, NC).

RESULTS

Within the full and subset samples, we found 7058 and 5139 men, respectively. A total of 3723 men were NHWs (78.97%), 1870 NHBs (11.01%), and 1465 Mexican Americans (10.02%) in the full sample with similar percentages in the subset sample (Table 1). Mean age in the full sample is 48.75 and subset sample is 48.87. In both full and subset samples, men had higher education (>12 years-high school, >30% some college), were overweight/obese (mean BMI > 28 kg/m^2), were never smokers (>47%), had prevalent diabetes (>12%) and hypertension (>43%), were physically active (>55%), and had moderate levels of alcohol consumption (mean >13 g) and total cholesterol (mean >191 mg/dL). Similar differences between the full and subset samples were observed when selected characteristic were stratified by CVD status (Supplemental Table 1).

In the full sample, only low T was associated with an increased prevalence of CVD (OR = 1.26, 95% CI, 1.02–1.57) after adjusting for CVD risk factors (Table 2). In the subset sample, after adjusting for the same CVD risk factors plus estradiol and SHBG levels, low T (OR = 1.57, 95% CI, 1.17–2.11), quintiles of total T (Q_1 vs Q_5, OR = 2.25, 95% CI, 1.01–5.01, P_{trend} = 0.02), low calculated free T (OR = 1.53, 95% CI, 1.10–2.17) and quintiles of calculated free T (Q_1 vs Q_5, OR = 1.59, 95% CI, 0.68–3.72, P_{trend} = 0.03) were associated with an increased prevalence of CVD (Table 2).

In both full and subset samples, low T was significantly associated with an increased prevalence of MI (OR = 1.40, 95% CI, 1.03–1.89, and OR = 1.72, 95% CI, 1.08–2.75, respectively) (Table 3). In both full and subset samples, low T was associated with an increase prevalence of HF (OR = 1.51, 95% CI, 1.09–2.10, and OR = 1.74, 95% CI, 1.08–2.85, respectively). Similar inverse associations with HF were found with quintiles of total T (Q_1 vs Q_5) in the full (Q_1 vs Q_5, OR = 1.92, 95% CI, 1.09–3.51, P_{trend} = 0.03) and subset samples (Q_1 vs Q_5, OR = 3.34, 95% CI, 1.22–9.13, P_{trend} = 0.004). For HF and CAD, only in the subset samples we found that a continuous increment of total T reduced prevalence of these diseases (Table 3).

Among NH-White men, both full and subset samples shows that only low T was significantly associated with an increased prevalence of CVD (OR = 1.34, 95% CI, 1.04–1.73, and OR = 1.73, 95% CI, 1.26–2.38, respectively) (Table 4). Only in the subset sample, low calculated free T (OR = 1.67, 95% CI, 1.10–2.49) and quintiles of calculated free T (Q_1 vs $Q_{5,}$ OR = 1.80, 95% CI, 0.66–4.95, P_{trend} = 0.03) were associated with an increased prevalence of CVD. Among Mexican American men, only in the subset sample we found that with continuous increments of total T (OR = 0.71, 95% CI, 0.51–0.97, P_{trend} = 0.03) and calculated free T (OR = 0.73, 95% CI, 0.58–0.92, P_{trend} = 0.01) there were reduced associations with prevalence of CVD. Low calculated free T (OR = 2.55, 95% CI, 1.35–4.81) and quintiles of calculated free T (Q_1 vs $Q_{5,}$ OR = 1.57, 95% CI, 0.27–8.97, P_{trend} = 0.03) were associated with an increased prevalence of CVD. In general, among NH-Black men, there were no significant associations (Table 4).

DISCUSSION

To our knowledge, the novelty of this study is the quantification of the associations of total T and calculated free T with CVD, and its specific disease outcomes (MI, HF, CAD), among a US nationally representative sample of NH-White, NH-Black, and Mexican American men. Our findings showed that low T or T deficiency, low calculated free T, total T (Q_1 vs Q_5), and calculated free T (Q_1 vs Q_5) were associated with an increased prevalence of CVD after adjusting for CVD-risk factors plus estradiol and SHBG (subset sample). Similarly, low T was associated with an increased prevalence of MI and HF, and the continuous increment of total T was associated with a decreased prevalence of CAD and HF. In general, the direction and significance of these associations were consistent among NH-White and Mexican American men, but not Black men.

Three meta-analyses of observational studies reported that low levels of total T were associated with an increased incidence of CVD and CVD mortality in 2011 [28–30], but others have not [31–33]. Subsequently, a 2018 larger meta-analysis of observational studies confirmed these inverse associations [34]. Yet, none of these meta-analyses conducted specific analysis among NH-Black and Mexican American men. Our findings among the overall population (NH-White, NH-Black and Mexican American) full sample are consistent with the results of these meta-analyses in relation to the negative association between low levels of T and higher risk of CVD. Furthermore, the largest studies included in the 2018 meta-analysis [34] were conducted mainly among NH-White men (between 2084 and 3637 participants included in the independent studies) [7, 35–40]. In our study, the largest racial group was NH-White men (n = 3723), and our findings in this group were similar to those reported by the previous meta-analyses [28–30, 34].

Differences in the levels of total T among adult and adolescent NH-White, NH-Black and Mexican American men have been previously noted and found that Mexican American adult and adolescent men had higher levels of total T than their counterparts NH-White and NH-Blacks [18, 41]. These previous findings have the potential to provide insight to our study observations as we only found significant associations between low levels of T and CVD among Mexican American and NH-White men.

Similar to the previous studies of total T, low free T has been linked with an increased risk of CVD and CVD mortality [7, 30]. However, these previous free T studies did not conduct specific analysis on NH-Black and Hispanic men. In a meta-analysis of 7 prospective studies, low free T was associated with an increased risk of CVD among healthy, middled aged and older men [30]. Our findings are consistent with these previous studies [30] as we found that low calculated free T was associated with an increased prevalence of CVD in the overall population ($n = 5139$) and among NH-White men ($n = 2688$, which included middle aged and older adults). In our study, a similar significant association was observed among Mexican Americans (middled aged and older men, $n = 1465$).

There is a considerable debate regarding whether calculated free T is a stronger indicator of T deficiency/hypogonadism diagnosis [13, 24] than total T particularly in view of recent reports of stronger associations of low free T (compared with low total T) with CVD mortality [7] and prostate cancer [42]. However, our findings with low T and low calculated free T among NH-White and Mexican American men do not support that contention.

What remains to be determined is the observation of inconsistent associations between T deficiency and CVD among Mexican American and NH-Black men, who have the highest prevalence for diabetes, obesity, and metabolic syndrome [19, 43] and which are considered among the strongest risk factors for T deficiency and risk of CVD [2, 19, 44], after taking into account these comorbidities. These inconsistent associations may suggest that other biological pathway (e.g., inflammation pathway [45, 46]) may influence the interplay between T deficiency and CVD by race/ethnicity.

Our study has strengths. NHANES includes a nationally representative sample of the civilian non-institutionalized US population; therefore, the findings of this study can be generalized to the US population. Furthermore, NHANES adheres to a rigorous protocol of quality control procedures for the collection of the outcomes of interests, exposures and potential confounding factors analyzed and adjusted in this study. In this study, we mutually adjusted for total T, SHBG, and estradiol. The scope of this investigation is not to demonstrate whether calculated free T is better than total T or viceversa, but rather to follow current guidelines from several societies suggesting the use of free T as a confirmatory marker in cases of borderline low total T [14].

Despite these strengths, the current study has limitations that may influence interpretation of the results. First, we conducted a cross-sectional study that precludes the investigation of a temporal investigation between T and CVD. Furthermore, we relied on a single measurement of sex steroid hormones. This limits our ability to make a strong causal inference between T levels and CVD outcomes. It is possible that- at least in some men- CVD and associated pharmacotherapy resulted in decreased T levels. In addition, due to limited power sample size, we couldn't conduct stroke specific analysis as the validity of the models were questionable. Second, the storage of serum or plasma in collection tubes after the centrifugation procedure can influence the measured total T process, and the ethylenediaminetetraacetic acid in collection tubes can influence SHBG, and subsequently these two can affect the calculation for free T [24]. Estradiol and SHBG were not available in all the NHANES cycles (2011–2012); therefore, two different datasets were created, full (1988–1991, 1999–2004, and 2011–2016) and subset samples (1988–1991, 1999–2004, and 2013–2016). The use of these two samples will allow comparison with future studies where estradiol and SHBG may or may not be available. Third, the latter NHANES waves (2011–2016) measured total T with HPLC tandem mass spectrophotometry, but the previous ones (1988–1991, 1999–2004) used electrochemiluminescence immunoassays. Fourth, the cut-off points to define T deficiency and calculated low free T may not fully or precisely capture their biological effect. Fifth, NHANES had no information related to testosterone replacement therapy, which could have influenced our findings and others have suggested caution about the use of testosterone therapy in the prevention of CVD [47]. Sixth, although we adjusted for several CVD risk factors, there remains the possibility of residual confounding. Finally, future studies with available data should explore in depth the role of population genomics and how genomic variation could influence the association between testosterone and CVD in different racial and ethnic groups [48, 49] that could provide insight about our findings with Mexican American men.

In summary, the results of this study confirm and elaborate on the observation that men with low levels of total T and calculated free T had an increased prevalence of CVD. Similarly, T deficiency was associated with an increased prevalence of MI and HF, and the continuous increment of total T was associated with a decreased prevalence of CAD and HF. In general, the direction and significance of these associations were consistent among NH-White and Mexican American men, but not Black men. Future studies with prospective designs and larger sample sizes of NH-Black and Mexican American men are required to confirm these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY

All data generated or analyzed in this study was provided by the National Center for Health Statistics (NCHS) of the US Centers for Disease Control and Prevention (CDC)- https://www.cdc.gov/nchs/nhanes/index.htm. For further data inquiries, please contact the corresponding author of this paper (DSL).

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Table 1.

Selected characteristics of the U.S. population of adult men 20 y and older in the full and subset samples in NHANES 1988–1991, 1999–2004, 2011–2016.

Subject characteristics	*Full Sample*[a,b] *(N = 7058) N (%) or Mean (SD)*	*Subset Sample*[b,c] *(N = 5139) N (%) or Mean (SD)*
Age, years	48.75 (17.92)	48.87 (17.99)
Age category		
20–39	2468 (38.53)	1804 (39.45)
40–49	1073 (18.21)	794 (18.13)
50–59	1024 (18.13)	745 (17.43)
≥60	2239 (25.13)	1647 (24.99)
Race and ethnicity		
Non-Hispanic White	3723 (78.97)	2688 (78.78)
Non-Hispanic Black	1870 (11.01)	1267 (11.02)
Mexican American	1465 (10.02)	1184 (10.20)
Education		
<9th grade	768 (6.20)	598 (6.93)
9th-11th grade	1150 (10.59)	853 (10.25)
High school/GED	1642 (22.54)	1172 (22.87)
Some college	2009 (30.21)	1453 (30.14)
College graduate or above	1479 (30.46)	1055 (29.81)
Body mass index (BMI), kg/m^2	28.78 (6.05)	28.37 (5.97)
Obesity		
Obese (BMI ≥ 30)	2358 (35.01)	1655 (34.86)
Non-Obese (BMI < 30)	4700 (64.99)	3484 (65.14)
Cigarette smoking		
Never	3078 (47.87)	2214 (47.95)
Former	2120 (29.06)	1345 (29.25)
Current	1860 (23.08)	1580 (22.80)
Diabetes		
No	5956 (87.80)	4339 (87.26)
Yes	1102 (12.20)	800 (12.74)
Alcohol consumption, g	13.39 (30.84)	13.49 (31.38)
Physical activity		
No	3836 (44.58)	2908 (45.43)
Yes	3222 (55.42)	2231 (54.57)
Total cholesterol, mg/ dL	191.49 (43.37)	193.20 (44.11)
Hypertension		
No	4685 (68.86)	3470 (69.24)
Yes	2373 (31.14)	1669 (30.76)

Subject characteristics	*Full Sample*[a,b] *(N = 7058) N (%) or Mean (SD)*	*Subset Sample*[b,c] *(N = 5139) N (%) or Mean (SD)*
Cardiovascular Disease (CVD)		
No	6262 (91.37)	4558 (91.26)
Yes	796 (8.63)	581 (8.74)
Total testosterone, ng/ mL[d]	4.02 (3.97, 4.07)	4.18 (4.12, 4.23)
Testosterone deficiency, (≤3.0 ng/mL)		
No	5420 (76.02)	4081 (78.20)
Yes	1638 (23.97)	1058 (21.80)
Non-Hispanic White with testosterone deficiency	922 (13.06)	590 (11.48)
Non-Hispanic Black with testosterone deficiency	386 (5.47)	222 (4.32)
Mexican American with testosterone deficiency	330 (4.68)	246 (4.79)
Calculated free testosterone, ng/mL[d]	ND	0.075 (0.073, 0.076)
Low calculated free testosterone (≤0.065 ng/mL)		
No	ND	3286 (63.12)
Yes	ND	1841 (36.89)
Total estradiol, pg/mL[d]	ND	26.98 (26.65, 27.30)
SHBG, nmol/L[d]	ND	38.48 (37.94, 39.03)

NHANES National Health and Nutrition Examination Survey, *SE* standard error, *GED* General Education Development, *kg* kilograms, *m*

Meter, *g* grams, *mg* milligram, *dL* deciliter, *CVD* cardiovascular disease, *ng* nanograms, *mL* milliliter, *pg* picogram, *nmol* nanomolar, *SHBG* sex hormone binding globulin, *ND* not determined.

[a]NHANES III (Phase I), 1999–2004, 2011–2016.

[b]Sampling weights were applied.

[c]NHANES III (Phase I), 1999–2004, 2013–2016; estradiol and SHBG data was unavailable in 2011–2012.

[d]Geometric mean, (95% confidence interval).

Table 2.

Multivariable associations of total and calculated free testosterone (T) with cardiovascular diseases in the full and subset samples in NHANES III Phase I (1988–1991), 1999–2004, and 2011–2016.

	Full Sample [a]			***Subset Sample*** [a,b]		
Sex steroid measurement	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Total testosterone (ng/mL)	0.94	0.88–1.02	0.127	0.85	0.72–1.00	0.054
Testosterone deficiencyc						
No	1.0	Reference		1.0	Reference	
Yes	1.26	1.02–1.57	0.032	1.57	1.17–2.11	0.003
Total testosterone quintiles, ng/mL						
Q1 (0.01–2.06)	1.13	0.75–1.71	0.096[e]	2.25	1.01–5.01	0.020[e]
Q2 (2.07–3.30)	0.85	0.55–1.32		1.96	1.00–3.83	
Q3 (3.31–4.34)	0.96	0.62–1.50		1.39	0.72–2.69	
Q4 (4.35–5.68)	0.75	0.47–1.20		1.19	0.74–1.91	
Q5 (>5.68)	1.0	Reference		1.0	Reference	
Calculated free testosterone, ng/mL	ND			0.99	0.91–1.10	0.837
Low calculated free testosterone[d]						
No	ND	ND	ND	1.0	Reference	0.019
Yes				1.53	1.10–2.17	
Calculated free testosterone quintiles, ng/mL						
Q1 (≤0.050)	ND	ND	ND	1.59	0.68–3.72	0.034[e]
Q2 (0.051–0.068)				1.06	0.51–2.19	
Q3 (0.069–0.087)				0.98	0.46–2.10	
Q4 (0.088–0.114)				0.78	0.36–1.68	
Q5 (>0.114)				1.0	Reference	

OR odds ratios, *CI* confidence interval, *ND* not determined, *ng* nanogram, *mL* milliliter.

[a] Weighted multivariable-adjusted logistic regression models. Full sample was adjusted for age, body mass index (weight in kilograms divided by height in meters squared), total alcohol consumption (grams), smoking status (never, past, or current), education level, physical activity (moderate and vigorous), total cholesterol (mg/dL), diabetes, and hypertension.

[b] Weighted multivariable-adjusted logistic regression models. Subset sample was adjusted for similar risk factors above plus estradiol and SHBG levels.

[c] Low T or Testosterone deficiency (≤3.0 ng/mL).

[d] Low calculated free testosterone (≤0.065 ng/mL).

[e] P trend.

Table 3.

CVD specific analysis: Multivariable associations of total and calculated free testosterone with myocardial infarction, heart failure, and coronary heart disease in the full and subset samples in NHANES III Phase I (1988–1991), 1999–2004, 2011–2016.

	Total Sample [a]			*Subset Sample* [a,b]		
Sex steroid measurement	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Myocardial Infarction						
Total testosterone (ng/mL)	0.97	0.88–1.08	0.669	0.98	0.81–1.19	0.851
Testosterone deficiency[c]						
No	1.0	Reference		1.0	Reference	
Yes	1.40	1.03–1.89	0.03	1.72	1.08–2.75	0.02
Total testosterone quintiles, ng/mL						
Q1 (0.01–2.06)	0.88	0.46–1.71	0.471[e]	0.94	0.36–2.43	0.442[e]
Q2 (2.07–3.30)	1.19	0.74–1.92		1.19	0.53–2.69	
Q3 (3.31–4.34)	0.70	0.40–1.24		0.65	0.30–1.42	
Q4 (4.35–5.68)	0.76	0.44–1.31		0.64	0.35–1.17	
Q5 (>5.68)	1.0	Reference		1.0	Reference	
Calculated free testosterone, ng/mL	ND			1.02	0.99–1.04	0.211
Low calculated free testosterone[d]						
No	ND	ND	ND	1.0	Reference	0.290
Yes				1.31	0.79–2.18	
Calculated free testosterone quintiles, ng/mL						
Q1 (≤0.050)	ND	ND	ND	0.71	0.27–1.92	0.824[e]
Q2 (0.051–0.068)				0.81	0.29–2.21	
Q3 (0.069–0.087)				0.62	0.23–1.66	
Q4 (0.088–0.114)				0.52	0.19–1.41	
Q5 (>0.114)				1.0	Reference	
Heart Failure						
Total testosterone (ng/mL)	0.90	0.81–1.00	0.064	0.78	0.64–0.96	0.02
Testosterone deficiency[c]						

Sex steroid measurement	Total Sample [a]			Subset Sample [a,b]		
	OR	95% CI	*P*	OR	95% CI	*P*
No	1.0	Reference		1.0	Reference	
Yes	1.51	1.09–2.10	0.014	1.74	1.08–2.82	0.024
Total testosterone quintiles, ng/mL						
Q1 (0.01–2.06)	1.92	1.05–3.51	0.030[e]	3.34	1.22–9.13	0.004[e]
Q2 (2.07–3.30)	1.46	0.83–2.56		1.95	0.82–4.63	
Q3 (3.31–4.34)	0.99	0.53–1.85		1.23	0.44–3.44	
Q4 (4.35–5.68)	1.21	0.67–2.18		1.94	0.51–2.12	
Q5 (>5.68)	1.0	Reference		1.0	Referent	
Calculated free testosterone, ng/mL	ND	ND	ND	1.05	0.95–1.13	0.339
Low calculated free testosterone[d]						
No	ND	ND	ND	1.0	Reference	0.843
Yes				0.94	0.51–1.73	
Calculated free testosterone quintiles, ng/mL						
Q1 (≤0.050)	ND	ND	ND	1.02	0.32–3.21	0.552[e]
Q2 (0.051–0.068)				0.64	0.19–2.14	
Q3 (0.069–0.087)				0.85	0.29–2.43	
Q4 (0.088–0.114)				0.60	0.19–1.92	
Q5 (>0.114)				1.0	Reference	
Coronary Heart Disease						
Total testosterone (ng/mL)	0.86	0.79–0.95	0.002	0.84	0.75–0.95	0.01
Testosterone deficiency[c]						
No	1.0	Reference		1.0	Reference	
Yes	1.41	0.99–2.03	0.609	1.10	0.67–1.87	0.694
Total testosterone quintiles, ng/mL						
Q1 (0.01–2.06)	2.17	1.02–4.58	0.059[e]	1.93	0.69–5.36	0.285[e]
Q2 (2.07–3.30)	1.99	1.11–3.56		1.62	0.76–3.44	
Q3 (3.31–4.34)	1.55	0.78–3.09		1.47	0.62–3.52	
Q4 (4.35–5.68)	1.77	0.99–3.13		1.61	0.81–3.19	

Sex steroid measurement	Total Sample [a]			Subset Sample [a,b]		
	OR	**95% CI**	***P***	**OR**	**95% CI**	***P***
Q5 (>5.68)	1.0	Reference		1.0	Reference	
Calculated free testosterone, ng/mL	ND	ND	ND	0.93	0.85–1.02	0.100
Low Calculated free testosterone deficiency						
No	ND	ND	ND	1.0	Reference	0.397
Yes				1.28	0.72–2.27	
Calculated free testosterone Quintiles, ng/mL						
Q1 (≤0.050)	ND	ND	ND	2.98	0.87–10.27	0.179[e]
Q2 (0.051–0.068)				2.09	0.63–6.92	
Q3 (0.069–0.087)				1.96	0.60–6.36	
Q4 (0.088–0.114)				2.41	0.69–8.44	
Q5 (>0.114)				1.0	Reference	

OR odds ratios, *CI* confidence interval, *ND* not determined, *ng* nanograms, *mL* milliliter, *MI* myocardial infarction, *HF* heart failure, *CAD* coronary heart disease.

[a] Weighted multivariable-adjusted logistic regression models. Full sample was adjusted for age, body mass index (weight in kilometers divided by height in meters squared), total alcohol consumption (grams), smoking status (never, past, or current), education level, physical activity (moderate and vigorous), total cholesterol (mg/dL), diabetes, and hypertension.

[b] Weighted multivariable-adjusted logistic regression models. Subset sample was adjusted for similar risk factors above plus estradiol and SHBG levels.

[c] Low T or Testosterone deficiency (≤3.0 ng/mL).

[d] Calculated Free testosterone deficiency (≤0.065 ng/mL).

[e] P trend.

Table 4.

Race and Ethnicity: Multivariable associations of total and calculated free testosterone with cardiovascular diseases in in the full and subset samples in NHANES III Phase I (1988–1991), and the 1999–2004, 2011–2016.

	Full Sample [a]			*Subset Sample* [a,b]		
Sex steroid measurement	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Non-Hispanic-White						
Total testosterone (ng/mL)	0.93	0.85–1.02	0.114	0.91	0.75–1.12	0.389
Testosterone deficiency[c]						
No	1.0	Reference		1.0	Reference	
Yes	1.34	1.04–1.73	0.024	1.73	1.26–2.38	0.0009
Total testosterone quintiles, ng/mL						
Q1 (0.01–2.06)	1.40	0.80–2.43	0.074[e]	1.83	0.81–4.16	0.05[e]
Q2 (2.07–3.30)	1.67	1.09–2.55		1.68	0.33–3.31	
Q3 (3.31–4.34)	1.21	0.73–1.99		1.20	0.33–2.40	
Q4 (4.35–5.68)	1.30	0.84–2.00		1.09	0.24–1.84	
Q5 (>5.68)	1.0	Reference		1.0	Reference	
Calculated free testosterone, ng/mL	ND			0.99	0.88–1.10	0.860
Low calculated free testosterone[d]						
No	ND	ND	ND	1.0	Reference	0.02
Yes				1.67	1.10–2.49	
Calculated free testosterone Quintiles, ng/mL						
Q1 (≤0.050)	ND	ND	ND	1.80	0.66–4.95	0.03[e]
Q2 (0.051–0.068)				1.17	0.50–2.75	
Q3 (0.069–0.087)				1.03	0.41–2.58	
Q4 (0.088–0.114)				0.80	0.32–2.02	
Q5 (>0.114)				1.0	Reference	
Non-Hispanic- Black						
Total testosterone (ng/mL)	1.05	0.94–1.16	0.388	1.10	0.92–1.31	0.311
Testosterone deficiency[c]						

	Full Sample [a]			Subset Sample [a,b]		
Sex steroid measurement	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
No	1.0	Reference		1.0	Reference	
Yes	0.85	0.50–1.43	0.533	0.93	0.49–1.76	0.823
Total testosterone quintiles, ng/mL						
Q1 (0.01–2.06)	0.66	0.26–1.43	0.175[e]	0.58	0.18–1.89	0.195[e]
Q2 (2.07–3.30)	0.82	0.43–1.56		0.69	0.38–1.46	
Q3 (3.31–4.34)	0.75	0.40–1.41		0.62	0.57–1.48	
Q4 (4.35–5.68)	1.23	0.73–2.09		1.05	0.53–1.99	
Q5 (>5.68)	1.0	Reference		1.0	Reference	
Calculated free testosterone, ng/mL	ND	ND	ND	1.05	0.95–1.13	0.339
Low calculated free testosterone[d]						
No	ND	ND	ND	1.0	Reference	0.143
Yes				0.62	0.32–1.18	
Calculated free testosterone quintiles, ng/mL						
Q1 (≤0.050)	ND	ND	ND	0.53	0.15–1.88	0.196[e]
Q2 (0.051–0.068)				0.56	0.18–1.73	
Q3 (0.069–0.087)				1.02	0.27–3.77	
Q4 (0.088–0.114)				0.84	0.29–2.41	
Q5 (>0.114)				1.0	Reference	
Mexican American						
Total testosterone (ng/mL)	0.93	0.76–1.14	0.501	0.71	0.51–0.97	0.03
Testosterone deficiency[c]						
No	1.0	Reference		1.0	Reference	
Yes	1.18	0.62–2.24	0.609	1.50	0.67–3.33	0.318
Total testosterone quintiles, ng/mL						
Q1 (0.01–2.06)	2.15	0.71–6.51	0.365[e]	3.75	0.95–14.85	0.154[e]
Q2 (2.07–3.30)	1.11	0.47–2.60		1.07	0.19–5.98	
Q3 (3.31–4.34)	1.02	0.41–2.56		0.95	0.21–4.31	
Q4 (4.35–5.68)	1.26	0.48–3.30		1.13	0.28–4.64	

	Full Sample [a]			Subset Sample [a,b]		
Sex steroid measurement	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Q5 (>5.68)	1.0	Reference		1.0	Reference	
Calculated free testosterone, ng/mL[d]	ND	ND	ND	0.73	0.58–0.92	0.01
Low calculated free testosterone[d]						
No	ND	ND	ND	1.0	Reference	0.004
Yes				2.55	1.35–4.81	
Calculated free testosterone quintiles, ng/mL						
Q1 (≤0.050)	ND	ND	ND	1.57	0.27–8.97	0.03[e]
Q2 (0.051–0.068)				0.57	0.35–3.32	
Q3 (0.069–0.087)				0.37	0.23–2.10	
Q4 (0.088–0.114)				1.00	0.20–1.59	
Q5 (>0.114)				1.0	Reference	

Interaction between race/ethnicity and total testosterone and its association with CVD: full sample $P_{\text{interaction}}$ = 0.344; subset sample $P_{\text{interaction}}$= 0.842.

Interaction between race/ethnicity and testosterone and association with CVD: full sample $P_{\text{interaction}}$ = 0.252; subset sample: $P_{\text{interaction}}$ = 0.784.

OR odds ratios, *CI* confidence interval, *ND* not determined, *ng* nanograms, *mL* milliliter, *NH* non-Hispanic.

[a] Weighted multivariable-adjusted logistic regression models. Full sample was adjusted for age, body mass index (weight in kilometers divided by height in meters squared), total alcohol consumption (grams), smoking status (never, past, or current), education level, physical activity (moderate and vigorous), total cholesterol (mg/dL), diabetes, and hypertension.

[b] Weighted multivariable-adjusted logistic regression models. Subset sample was adjusted for similar risk factors above plus estradiol and SHBG levels.

[c] Low T or Testosterone deficiency (≤3.0 ng/mL).

[d] Calculated free testosterone deficiency (≤0.065 ng/mL).

[e] P trend.