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Association between endogenous sex steroid hormones and inflammatory biomarkers in US men

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Abstract

Sex steroid hormones and inflammatory biomarkers are both associated with the development and progression of chronic diseases, but their interrelationship is relatively uncharacterized. We examined the association of sex hormones and sex hormone binding globulin (SHBG) with biomarkers of inflammation, C-reactive protein (CRP) and white blood cell (WBC) count. The study included data from 809 adult men in the National Health and Nutrition Examination Survey 1999–2004. Geometric means and 95% confidence intervals were estimated separately for CRP and WBC concentrations by sex steroid hormones and SHBG using weighted linear regression models. Higher concentrations of total (slope per 1 quintile in concentration, −0.18; P-trend, 0.001) and calculated free (slope, −0.13; P-trend, 0.03) testosterone were statistically significantly associated with lower concentrations of CRP, but not with WBC count. Men in the bottom quintile of total testosterone (≤3.3 ng/mL), who might be considered to have clinically low testosterone, were more likely to have elevated CRP (≥ 3 mg/L) compared to men in the top four quintiles (OR, 1.61; 95% CI, 1.00 – 2.61). Total and calculated free estradiol (E2) were positively associated with both CRP (Total E2: slope, 0.14; P-trend, <0.001; Free E2: slope, 0.15; P-trend, <0.001) and WBC (Total E2: slope, 0.02; P-trend, 0.08; Free E2: slope, 0.02; P-trend, 0.02) concentrations. SHBG concentrations were inversely associated with WBC count (slope, −0.03; P-trend, 0.04), but

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DISCLOSURES

None of the authors has a conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors participated in the design and conduct of the study. KT analyzed the data. KT and EP wrote the manuscript, but all authors read, made substantial contributions and approved the final version.

not with CRP. These cross-sectional findings are consistent with the hypothesis that higher androgen and lower estrogen concentrations may have an anti-inflammatory effect in men.

Keywords

testosterone; estradiol; C-reactive protein; white blood cell; cross-sectional study

INTRODUCTION

Endogenous androgen concentrations decline with increasing age in men after puberty (Feldman et al. 2002; Rohrmann et al. 2011). In parallel, meta-analyses have shown that low testosterone concentrations in men are associated with increased risk of all-cause and cardiovascular disease death (Araujo et al. 2011), and increased risk of type 2 diabetes development (Ding et al. 2006). Sex steroid hormones have long been hypothesized to be related to prostate cancer development, mainly due to the growth promoting activities of testosterone and its metabolites (Platz and Giovannucci 2004), although circulating concentrations are not clearly associated with the risk of prostate cancer in epidemiologic studies (Roddam et al. 2008).

An increase in inflammatory biomarkers with age has been reported, and C-reactive protein (CRP), a sensitive but not specific biomarker of inflammation, has been independently associated with increased risk of coronary heart disease and stroke development and death (Kaptoge et al. 2010). Mechanistic evidence supports that intraprostatic inflammation may contribute to prostate carcinogenesis (Nelson et al. 2004), but epidemiologic studies of sexually transmitted infections, clinical prostatitis, and genetic or circulating markers of inflammation and risk of prostate cancer have reported inconsistent results (Platz and De Marzo 2004).

Little is known about if and how steroid sex hormone and inflammation pathways may interact to influence the aging process or the development and progression of chronic diseases, including cardiovascular disease and prostate cancer, in men. Two randomized controlled trials of androgen replacement therapy in hypogonadal men reported a decrease in the concentration of pro-inflammatory cytokines in the active therapy arm (Malkin et al. 2004; Kalinchenko et al. 2010), but other trials did not confirm these findings (Ng et al. 2002; Singh et al. 2002; Kapoor et al. 2007; Nakhai-Pour et al. 2007). A few epidemiologic studies have evaluated the cross-sectional association between sex steroid hormones and inflammatory biomarkers in men, but had inconsistent results (Laaksonen et al. 2003; Van Pottelbergh et al. 2003; Bhatia et al. 2006; Maggio et al. 2006; Nakhai Pour et al. 2007; Tang et al. 2007; Maggio et al. 2009; Schneider et al. 2009; Kaplan et al. 2010; Kupelian et al. 2010; Brand et al. 2012; Zhang et al. 2012). Moreover, some of the latter studies did not adjust for important confounders (e.g., smoking and obesity) (Laaksonen et al. 2003; Van Pottelbergh et al. 2003; Bhatia et al. 2006; Tang et al. 2007; Kaplan et al. 2010), and very few studies mutually adjusted for other sex hormones (Nakhai Pour et al. 2007; Zhang et al. 2012). Therefore, the aim of this study was to investigate the cross-sectional association between endogenous sex steroid hormones (total testosterone, calculated free testosterone, total estradiol, calculated free estradiol and androstanediol glucuronide, a metabolite of dihydrotestosterone), sex hormone binding protein (SHBG) and biomarkers of inflammation (CRP and white blood cell count [WBC]) in a large nationally representative sample of US men after carefully adjusting for all important confounders.

MATERIALS AND METHODS

Study population

The National Health and Nutrition Examination Survey (NHANES) is a program of studies undertaken by the National Center for Health Statistics (NCHS) of the United States (US) Centers for Disease Control and Prevention (CDC) to assess the health and nutritional status of adults and children in the US (1994). It is based on a stratified multistage probability design and is weighted to represent the total US civilian, non-institutionalized population. The NHANES includes an interview, and an examination component that includes blood collection. Investigators are allowed to access surplus sera for approved studies.

The current study included data from male participants in the 1999–2000 (n = 9,965), 2001–2002 (n = 11,039) and 2003–2004 (n = 10,122) NHANES cycles. Endogenous sex hormones were measured from stored surplus serum samples by the study investigators in 1,520 males, who were a stratified random sample of participants in the morning examination sessions of each cycle. Morning sample participants were chosen to reduce extraneous variation due to diurnal production of hormones. More information on the selection process of the 1,520 participants can be found elsewhere (Nyante et al. 2012).

Men younger than 20 years of age were excluded from this study (n = 529). Twenty-one men were excluded for having missing sex hormone measurements and an additional 9 men were excluded for having extreme hormone measurements. No men had missing measurements for the inflammatory biomarkers, but 4 men were excluded due to extreme values. The following cutpoints were used to determine extreme measurements based on a visual inspection of the distribution: testosterone = 50 ng/mL (n = 1), estradiol = 360 pg/mL (n = 1), androstanediol glucuronide > 40 ng/mL (n = 3), SHBG > 170 nmol/L (n = 4), CRP > 100 mg/L (n = 3), and WBC = $28.2 \cdot 10^9$ cells (n = 1). Men with a history of prostate cancer were also excluded because certain treatments may have affected hormone levels (n = 18). Finally, 130 men were excluded due to missing information on body mass index (BMI) (n = 18), waist circumference (n = 25), smoking status (n = 54), type 2 diabetes (n = 5), and alcohol consumption (n = 50), leaving 809 men in the final study sample. The baseline characteristics of these 809 men were similar to the characteristics of our sample size before the exclusions.

The NHANES program is approved by the Institutional Review Board of the NCHS at CDC. Informed consent was obtained from all participants. Addressing questions about hormones and men's health in NHANES was approved by the National Institutes of Health Office of Human Subjects Research and the NCHS Ethics Review Board at CDC.

Assessment of steroid sex hormones and SHBG

Serum hormone concentrations were measured in the laboratory of Dr. Nader Rifai at Children's Hospital in Boston, MA. Details on the blood draw, process, storage and shipping methods are provided elsewhere (Nyante et al. 2012). Testosterone, estradiol and SHBG were measured using the Elecsys 2010 system (Roche Diagnostics, Laval, QC, Canada), while androstanediol glucuronide was measured by enzyme immunoassay using the direct androstanediol glucuronide ELISA kit (ALPCO Diagnostics, Salem, NH). The laboratory technicians were blinded to the participant characteristics of the samples. The lower limits of detection of the assays were 0.02 ng/mL for testosterone, 5 pg/mL for estradiol, 0.33 ng/mL for androstanediol glucuronide, and 3 nmol/L for SHBG. One sample had a concentration below the limit of detection for testosterone and 10 samples for estradiol, which were assigned to half the limit of detection. Values of total testosterone below 3 ng/mL are considered as clinically low testosterone (hypogonadism). Twenty-one samples were assayed in duplicate for quality control purposes, and the coefficients of variation were 4.8%

for testosterone, 21.4% for estradiol, 9.7% for androstanediol glucuronide and 5.6% for SHBG. Free testosterone and free estradiol were calculated using published formulas with information for total testosterone, total estradiol, SHBG, and serum albumin (measured in NHANES) (Sodergard et al. 1982; Vermeulen et al. 1999). The testosterone to estradiol molar ratio was also calculated.

Assessment of inflammatory biomarkers

C-reactive protein concentrations were quantified by high-sensitivity latex-enhanced nephelometry on a BNII nephelometer (Dade Behring Diagnostics, Inc., Newark, DE). The lower limit of detection of the assay was 0.2 mg/L, and a value of 0.1 was assigned to values falling below this level (n = 12). White blood cell count was obtained through the Beckman Coulter sizing and counting method on a Beckman Coulter MAXM hematology analyzer (Block Scientific, Inc., Holbrook, NY). Detailed descriptions of blood collection and processing, the laboratory methods and quality control statistics for the inflammatory markers can be found elsewhere (Centers for Disease Control and Prevention 2000).

Assessment of covariates

Age, race/ethnicity, cigarette smoking, alcohol consumption, and physical activity were self-reported during the NHANES interviews. Waist circumference at the iliac crest, height and weight were measured during the examination. BMI was calculated as weight in kilograms divided by height in meters squared. The definition of diabetes was based on self-reported information of diabetes diagnosis and fasting plasma concentration of glucose. Glucose was measured in NHANES using the glucose hexokinase method with a Hitachi Model 704 multichannel analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). Serum cotinine, a metabolite of nicotine, was measured in NHANES by an isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. NHANES participants self-reported whether they had ever been told to take prescribed medication for high blood pressure and high cholesterol. Total cholesterol and triglyceride concentrations were measured in NHANES enzymatically, whereas HDL-cholesterol was measured with a heparin-manganese precipitation method in the 1999–2002 cycles, and with a direct immunoassay method in the 2003–2004 cycle. Glycosylated hemoglobin was measured in the 2002–2004 cycles using the principle of boronate affinity high performance liquid chromatography (Primus Corporation, Kansas City, MO).

Statistical analysis

Geometric means and 95% confidence intervals (CIs) for CRP and WBC concentrations were estimated by sex steroid hormones and SHBG using weighted linear regression models. CRP and WBC concentrations were transformed using the natural logarithm because they were right-skewed. Population quintiles of sex steroid hormones and SHBG concentrations were used, and inferences remained the same when tertiles or deciles of these distributions were applied. Non-parametric regression models and lowess plots of the inflammatory biomarkers versus endogenous sex hormones were also used to capture the best modeling approach, but no deviations from linearity were identified. The slope of the change in the natural logarithm CRP and WBC with increasing sex steroid hormones and SHBG concentration was estimated by entering quintiles values (e.g., 1, 2, 3, 4, 5) into the models as an ordinal variable, the coefficient for which was evaluated by the Wald test. Further analyses were conducted to estimate odds ratios (OR) and 95% CIs for the association between sex hormones or SHBG and elevated CRP concentrations at the suggested cut-point for cardiovascular risk (≥3 vs. <3 mg/L) using weighted logistic regression models.

Three multivariable linear regression models were built for each inflammatory biomarker and sex hormone pair. Model 1 was adjusted for age (continuous) and race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic including Mexican-American, other). Model 2 also included BMI (<18.5, 18.5 to 24.9, 25 to 29.9, ≥30 kg/m^2), waist circumference (continuous), cigarette smoking status (never, former, current smoker with <20 cigarettes/day, current smoker with ≥20 cigarettes/day), type 2 diabetes (no diabetes [no diagnosis and glucose <100 mg/dL], pre-diabetes [no diagnosis and glucose ≥100 to ≤125 mg/dL], diabetes [diagnosis or glucose >125 mg/dL]), alcohol consumption (never drinker, ≤1 drink/month, >1 drink/month to ≤1 drink/week, >1 drink/week to <1 drink/day, ≥1 drink/day) and leisure-time moderate or vigorous physical activity (0 times/week, <2 times/week, ≥2 to <7 times/week, ≥7 times/week). Model 3 was further mutually adjusted for testosterone, estradiol and SHBG because these hormones compete for binding to SHBG. Further adjustments for serum cotinine concentrations (not exposed to tobacco smoke [<0.035 ng/mL], passively exposed only [0.035 to 9.99 ng/mL], actively exposed [≥10 ng/mL] (Centers for Disease Control and Prevention 2000)), total cholesterol, HDL-cholesterol, triglycerides, glycosylated hemoglobin, use of medications to treat high blood pressure and cholesterol, and self-reported history of arthritis, myocardial infarction, heart failure, stroke, cancer and general health condition (excellent, very good, good, fair, poor) did not change the results, and were therefore not included in the final models. A sensitivity analysis was conducted to further limit the possible confounding by smoking by excluding current smokers (n = 184).

Stratified analyses were conducted by age (20 to 39 years, 40 to 59 years, ≥60 years), race/ethnicity, obesity (not obese by both BMI and waist circumference definitions [BMI <30 kg/m^2 and waist circumference <102 cm], obese by only one of the two definitions, obese by both definitions), type 2 diabetes (no, diabetes/pre-diabetes), general health condition (excellent/very good, good/fair/poor), and cotinine concentrations (no exposure, passive, active), because these variables are known to modulate inflammation and/or sex steroid hormone concentrations. Tests for interaction were carried out by using the ordinal sex steroid hormone and SHBG variables, ordinal or binary variables for the potentially modifying factors and their product terms. The statistical significance of the interaction terms was evaluated by the Wald test.

All p-values were two-sided; 0.05 was considered the cut-off for statistical significance, and all analyses were performed using survey data analysis commands in STATA version 10 (College Station, TX) to account for the complex NHANES sample survey design. The p-values from the interaction tests were not corrected for multiple hypothesis testing because the sex steroid hormones are highly inter-related, but they were interpreted in view of the 72 comparisons made (2 inflammatory biomarkers × 6 steroid hormones × 6 modifying variables).

RESULTS

Selected characteristics of the 809 adult men included in the analysis are presented in Table 1. After applying sampling weights, the mean age was 44 years, and 75% of the participants were white. The mean BMI was 28 kg/m^2, and the prevalence of obesity was 29% based on BMI (≥30 kg/m^2) and 39% based on waist circumference (≥102 cm). Twenty-five percent of the participants were current smokers, 23% abstained from alcohol, and 20% did some type of moderate or vigorous physical activity on average every day during the last month. Ten percent were diabetics, and a further 31% had a pre-diabetic condition. The geometric mean of CRP was 1.52 mg/L, and 27% of the participants had values corresponding to increased cardiovascular disease risk (≥3 mg/L). The mean values for WBC, sex steroid hormones and SHBG were within the normal range.

Geometric means and 95% CIs of CRP concentrations by quintiles of sex steroid hormones are shown in Table 2. Total (slope per 1 quintile increase in hormone concentration, −0.18; P-trend, 0.001) and calculated free (slope, −0.13; P-trend, 0.03) testosterone concentrations were inversely associated with CRP concentrations. The geometric mean of CRP was 1.08 mg/L in the top quintile of total testosterone and 2.18 mg/L in the bottom quintile after adjusting for age, race/ethnicity, BMI, waist circumference, diabetes, cigarette smoking, alcohol consumption, physical activity, total estradiol and SHBG. Estradiol (both total and calculated free) concentrations were positively associated with CRP (P-trend, <0.001). The geometric mean of CRP was 1.16 mg/L in the bottom quintile of total estradiol and 2.09 mg/L in the top quintile. SHBG concentration was inversely associated with CRP in models 1 (slope, −0.17; P-trend, <0.001) and 2 (slope, −0.08; P-trend, 0.04), but this association was no longer statistically significant after mutual adjustment for total testosterone and total estradiol (slope, 0.02; P-trend, 0.59). Concentrations of androstanediol glucuronide were not associated with CRP (P-trend, 0.30).

Table 3 shows the odds ratios and 95% CIs of elevated CRP (≥3 vs. <3 mg/L) concentrations by quintiles of sex steroid hormones. Compared to individuals in the bottom quintile of total (Q5 vs. Q1: OR, 0.17; 95% CI, 0.07 – 0.45; P-trend, <0.001) and calculated free (Q5 vs. Q1: OR, 0.27; 95% CI, 0.11 – 0.66; P-trend, 0.003) testosterone, men in the top quintile were less likely to have elevated CRP concentrations. Men in the bottom quintile of total testosterone, who might be considered to have clinically low testosterone, were more likely to have elevated CRP (OR, 1.61; 95% CI, 1.00 – 2.61) as compared to men in the top four quintiles. Higher concentrations of total (P-trend, 0.003) and calculated free (P-trend, 0.001) estradiol were positively associated with elevated CRP. SHBG and androstanediol glucuronide concentrations were not associated with elevated CRP.

Table 4 presents the geometric means and 95% CIs of WBC count by quintiles of sex steroid hormones with progressive adjustment for confounders. Total testosterone (P-trend, 0.45), calculated free testosterone (P-trend, 0.91) and androstanediol glucuronide (P-trend, 0.63) were not associated with WBC count. Calculated free estradiol was positively associated with WBC count (slope per 1 quintile increase in hormone concentration, 0.02; P-trend, 0.02), while the positive association between total estradiol and WBC was borderline significant (slope, 0.02; P-trend, 0.08). The molar ratio of total testosterone to estradiol was inversely associated with WBC count (slope, −0.02; P-trend, 0.04). SHBG concentrations were inversely associated with WBC (slope, −0.03; P-trend, 0.04).

Our findings for CRP were unchanged after excluding current smokers (data not shown). However, the associations of total testosterone and total estradiol with WBC count became significant after exclusion of current smokers: total testosterone was inversely associated with WBC count (slope per 1 quintile increase in hormone concentration, −0.03; P-trend, 0.05), and total estradiol was positively associated with WBC count (slope, 0.02; P-trend, 0.02).

We also stratified by factors that we hypothesized might modify the association between sex steroid hormones and CRP or WBC count. The results are presented in an Online Supplement (Supplemental Data Tables 1 and 2). Despite not being associated with CRP overall, the most significant interaction was observed for the association between androstanediol glucuronide and CRP concentrations by obesity (P-interaction, 0.007), where androstanediol glucuronide was inversely associated with CRP in obese men (slope per 1 quintile increase in hormone concentration, −0.13; P-trend, 0.003), and positively associated in men who were not obese (slope, 0.13; P-trend, 0.003). Otherwise, androstanediol glucuronide concentration was not associated with CRP within strata of any of the other factors evaluated. Significant interactions were also observed for the associations between

total estradiol (P-interaction, 0.03), calculated free estradiol (P-interaction, 0.02), SHBG (P-interaction, 0.05) and androstanediol glucuronide (P-interaction, 0.02) concentrations and CRP by race/ethnicity. Four statistically significant interactions were observed for the associations between sex steroid hormones and WBC count, and the strongest was between total testosterone and WBC by cotinine concentrations (P-interaction, 0.02), where the association was statistically significant inverse in passive smokers (slope, −0.053; P-trend, <0.001) but null in participants not exposed to tobacco smoke (slope, −0.015; P-trend, 0.54) and active smokers (slope, 0.021; P-trend, 0.30).

DISCUSSION

This cross-sectional nationally representative study of US males found modest statistically significant inverse associations for total and calculated free testosterone, and modest positive associations for total and calculated free estradiol with CRP concentration. Estradiol concentrations were also weakly positively associated with WBC count, and SHBG was weakly inversely associated with WBC. An association between testosterone and WBC count was not observed. These findings are consistent with the hypothesis that in men higher androgen concentration is anti-inflammatory, and higher estrogen concentration is pro-inflammatory. Further, the probability of elevated CRP concentrations (≥ 3 mg/L) decreased with higher total and calculated free testosterone concentrations, while the probability increased with higher total and calculated free estradiol concentrations. Men with clinically low testosterone may be able to reduce their chronic low-grade inflammation status, and thus achieve maximal benefit from lowering their future risk of cardiovascular disease and cancer, if they could raise their testosterone concentrations, for example, through weight loss and exercise. Other men who do not already have low testosterone might take steps to minimize their decline in testosterone as they age by avoiding weight gain and staying active.

The mechanisms explaining the associations between steroid sex hormones and inflammatory biomarkers are not completely understood. However, there is ample evidence supporting the immunosuppressive effect of androgens. The incidence of autoimmune diseases is higher in androgen-deficient men (Tengstrand et al. 2002). Studies have shown that the induction of hypogonadism in older men is followed by a significant increase in IL-6 concentrations (Khosla et al. 2002), a potent stimulator of inflammation, and that activation of the androgen receptor exerts a direct anti-inflammatory effect (Vignozzi et al. 2012). It has been suggested that the mechanisms for the immunosuppressive effect of androgens could be either a direct effect on the expression of inflammatory genes (Bellido et al. 1995; Asirvatham et al. 2006), or an indirect effect through inhibition of nuclear factor-kB activation (Vignozzi et al. 2012). Studies have also emphasized the role of estrogens in male physiology (MacDonald et al. 1979). Estradiol is the major biologically active estrogen, and about 80% is formed in adult men from the aromatization of testosterone primarily in the adipose tissue. It has been hypothesized that the potential positive association between estradiol and CRP is likely an effect on gene expression in the liver (Vongpatanasin et al. 2003). Elements of steroid sex hormone response have not been discovered in the promoter of the *CRP* gene, but a study has shown that estrogen can stimulate the transcription factor C/EBP-β, which is involved in CRP transcription (Kousteni et al. 2003).

Most prior cross-sectional studies have observed inverse associations between androgen concentrations and inflammatory biomarkers (Laaksonen et al. 2003; Bhatia et al. 2006; Tang et al. 2007; Schneider et al. 2009; Kaplan et al. 2010; Kupelian et al. 2010; Brand et al. 2012; Zhang et al. 2012). A recent study in Chinese men showed that lower concentrations of total and calculated free testosterone were associated with higher CRP concentration

(Zhang et al. 2012). Data from the Boston Area Community Health Survey also reported inverse associations between testosterone and CRP concentrations (Kupelian et al. 2010). In contrast, some other smaller cross-sectional studies did not observe statistically significant associations between testosterone and CRP concentrations, although their point estimates were indeed in the inverse direction (Maggio et al. 2006; Nakhai Pour et al. 2007). Fewer studies have looked at the association between androgen concentrations and WBC count. Total testosterone was inversely associated with WBC count (Tang et al. 2007; Schneider et al. 2009; Brand et al. 2012), but calculated free testosterone was not associated with WBC (Tang et al. 2007; Brand et al. 2012).

Randomized controlled trials of testosterone replacement therapy (TRT) in hypogonadal men have generally recruited small numbers of participants, and reported inconsistent results for the effect of testosterone on inflammatory biomarkers (Ng et al. 2002; Singh et al. 2002; Malkin et al. 2004; Kapoor et al. 2007; Nakhai-Pour et al. 2007; Kalinchenko et al. 2010). The two largest trials included 184 and 237 men, of which 105 and 113 men received TRT for 30 and 26 weeks, respectively (Nakhai-Pour et al. 2007; Kalinchenko et al. 2010). The first trial found a decrease in CRP, interleukin-1β (IL-1β), and tumor necrosis factor-α (TNFα) but no changes in IL-6 and IL-10 concentrations between the active treatment and placebo arms (Kalinchenko et al. 2010). The second trial observed no difference in CRP concentrations (Nakhai-Pour et al. 2007). Several other trials with 50 or fewer men in total each reported no differences in CRP concentrations between the TRT and placebo arms (Ng et al. 2002; Singh et al. 2002; Kapoor et al. 2007). Another small cross-over trial reported that testosterone induced reductions in TNFα, IL-1β, and an increase in IL-10 concentrations (Malkin et al. 2004).

In contrast to our findings, the majority of studies in the literature have not observed statistically significant associations between estradiol and inflammatory biomarkers in men, although several of them observed point estimates in the positive direction (Nakhai Pour et al. 2007; Maggio et al. 2009; Kupelian et al. 2010). The statistically significant positive association in our study was strengthened after mutual adjustment for total testosterone and SHBG, whereas no prior studies have adjusted for other sex hormones or SHBG (Van Pottelbergh et al. 2003; Nakhai Pour et al. 2007; Maggio et al. 2009; Kupelian et al. 2010; Zhang et al. 2012). It is likely that this mutual adjustment partly explains the stronger results in our study as compared to earlier reports, as total testosterone and estradiol compete for binding to SHBG, and seem to have opposite effects on the concentration of inflammatory biomarkers. A small randomized controlled trial of estrogen replacement therapy in prostate cancer patients showed an increase in CRP in the active treatment group versus the comparator group (orchidectomy) (Kovacs et al. 2005), a result consistent with the relationship we observed in NHANES, but another placebo-controlled trial observed null findings (Taxel et al. 2008).

We found that SHBG concentration was not associated with CRP after mutual adjustment for total testosterone and estradiol; a weak borderline significant inverse association was seen between SHBG and WBC count. Our results agree with a large cross-sectional study in China, where the association between SHBG and CRP was not significant after adjusting for total testosterone (Zhang et al. 2012). Several other cross-sectional studies have reported significant inverse associations between SHBG and CRP but without mutual adjustment (Laaksonen et al. 2003; Bhatia et al. 2006; Kupelian et al. 2010). A cross-sectional study in the United Kingdom observed lower WBC counts with higher SHBG concentration (Brand et al. 2012).

We found several weak but statistically significant interactions for the associations between steroid sex hormones and inflammatory biomarkers by race/ethnicity, obesity, diabetes, and

cotinine concentrations. These potential effect modifiers may modulate the general health condition of the participants, and affect the endogenous concentrations of inflammatory biomarkers and sex hormones. Obese men are known to have lower androgen concentrations compared to their normal-weight counterparts (Rohrmann et al. 2011). It is possible, of course, that some of these findings are due to chance given the large number of tests performed. Seventy-two tests of interaction were conducted, and 9 (13%) were significant at the conventional level of $p \leq 0.05$. The strongest suggestion of an interaction was the inverse association between androstanediol glucuronide and CRP concentrations in obese participants, while the association was positive in the non-obese. However, androstanediol glucuronide was not associated with CRP concentrations in the overall analysis. A recent Chinese cross-sectional study observed stronger inverse associations between total testosterone and CRP concentrations in individuals with a BMI of 27.5 kg/m^2 or greater (Zhang et al. 2012), an interaction we did not observe. Most previous studies have largely ignored potential effect modification, and future studies are needed to confirm our results in these subgroups.

The current study included a large sample of men; it is the first study of sex steroid hormones and inflammatory markers in men that is representative of the general US population; it measured several endogenous hormone concentrations and two inflammatory biomarkers using standard and highly reliable methods, and adjusted for a wide range of covariates including demographic, lifestyle and health variables, and it is one of the few studies that further mutually adjusted the results with other sex steroid hormones. In particular, our study used detailed adjustment for body fatness and smoking, which are strongly associated with both testosterone and CRP concentrations. However, our study is cross-sectional, and as such it cannot establish the temporal sequence of the observed associations. Thus, we cannot determine whether steroid sex hormones affect the concentration of inflammatory biomarkers, which is the prevalent hypothesis, or vice versa as a few reports suggest (Mauduit et al. 1998). We used a published formula to calculate free testosterone and free estradiol, which has been shown to correlate well with the values of the directly measured assays (Morley et al. 2002). Sex hormones and inflammatory biomarkers were measured once, which may not represent the men's usual levels; however, hormone measurements were all conducted using serum collected from morning blood draws, which eliminate potential variation in levels due to diurnal patterns. Future prospective studies with multiple biomarker measurements assessing also a larger panel of cytokines (e.g., tumor necrosis factor α, interleukin 6, 8, 10 and 17, etc.) are needed to shed light on the temporal association between sex hormones and inflammation.

In conclusion, our results suggest that total and calculated free testosterone are modestly inversely associated with CRP concentrations, and that total and calculated free estradiol are modestly positively associated with CRP and WBC. Steroid hormone and inflammation pathways may interact for the development or progression of chronic diseases, and investigators should examine these pathways jointly in future studies. In addition, our findings may imply a clinical benefit for men with low testosterone or men with normal testosterone who want to minimize their decline in testosterone as they age, if they could raise their testosterone concentrations through weight loss and exercise and thus achieve an anti-inflammatory effect.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SHBG	sex hormone binding globulin
CRP	C-reactive protein
WBC	white blood cell
E2	estradiol
OR	odds ratio
NHANES	National Health and Nutrition Examination Survey
NCHS	National Center for Health Statistics
CDC	Centers for Disease Control and Prevention
BMI	body mass index
CI	confidence intervals
HDL	high-density lipoprotein
TRT	testosterone replacement therapy
IL-1β	interleukin 1 beta
TNFα	tumor necrosis factor alpha
IL-6	interleukin six
IL-10	interleukin ten
SE	standard error

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Table 1

Selected characteristics of the U.S. population of adult men 20 years or older, NHANES 1999–2004

Characteristics	Unweighted sample size	Mean or percentage (SE)[†]
Age, years	809	44.3 (0.7)
Race/ethnicity (%)		
Non-Hispanic white	441	75.1 (2.3)
Non-Hispanic black	151	10.0 (1.1)
Hispanic	195	11.0 (1.9)
Other	22	3.9 (0.8)
Body mass index, kg/m^2	809	27.9 (0.2)
Waist circumference, cm	809	99.6 (0.6)
Cigarette smoking (%)		
Never	365	46.1 (2.2)
Former	280	28.7 (1.7)
Current, <20 cigs/day	83	9.1 (1.4)
Current, ≥20 cigs/day	101	16.1 (1.3)
Diabetes (%)[§]		
No	445	58.9 (2.5)
Pre-diabetes	263	31.4 (2.5)
Yes	101	9.6 (1.2)
Alcohol consumption (%)		
Never	214	22.6 (2.0)
≤1/month	193	24.2 (1.4)
>1/month - ≤1/week	178	22.9 (1.5)
>1/week - <1/day	170	23.9 (1.9)
≥1/day	54	6.4 (1.0)
Leisure-time physical activity (%)		
0 times/week	316	32.4 (1.8)
0.1–1.9 times/week	141	20.0 (1.9)
2–6.9 times/week	203	27.6 (2.3)
≥7 times/week	149	19.9 (1.7)
Total cholesterol, mg/dL	809	200.6 (197.5 – 203.6)
HDL cholesterol, mg/dL	809	46.3 (45.0 – 47.6)
Triglycerides, mg/dL	809	152.0 (140.2 – 163.9)
C-reactive protein, mg/L[‡]	809	1.52 (1.37 – 1.68)
White blood cells, ·10^9 cells/L[‡]	809	6.47 (6.34 – 6.60)
Neutrophils (%)	808	58.0 (0.3)
Eosinophils (%)	808	3.2 (0.1)
Basophils (%)	808	0.6 (0.02)
Lymphocytes (%)	808	29.3 (0.3)
Monocytes (%)	808	8.8 (0.08)

Characteristics	Unweighted sample size	Mean or percentage (SE)[†]
Total testosterone, ng/mL[‡]	809	4.60 (4.43 – 4.77)
Total estradiol, pg/mL[‡]	809	28.4 (27.2 – 29.7)
Sex hormone binding globulin, nmol/L[‡]	809	32.3 (30.8 – 33.9)
Androstanediol glucuronide, ng/mL[‡]	809	7.11 (6.80 – 7.43)
Calculated free testosterone, ng/mL[‡]	809	0.092 (0.088 – 0.096)
Calculated free estradiol, pg/mL[‡]	809	0.73 (0.69 – 0.76)
Total testosterone to estradiol molar ratio	809	0.18 (0.17 – 0.19)

Abbreviations: NHANES, National Health and Nutrition Examination Survey; SE, standard error; HDL, high-density lipoprotein

[†]Sampling weights were applied

[‡]Geometric mean (95% confidence intervals)

[§]Type 2 diabetes status was decided based on the self-reported diagnosis of diabetes and on the fasting plasma glucose concentrations: i) no diabetes (no diagnosis and glucose <100 mg/dL), ii) pre-diabetes (no diagnosis and glucose ≥100 to ≤125 mg/dL), and iii) diabetes (diagnosis or glucose >125 mg/dL)

Table 2

Geometric means and 95% confidence intervals of C-reactive protein (mg/L) concentrations by quintiles of sex steroid hormones in the U.S. population of adult men 20 years or older, NHANES 1999–2004

	Model 1[†]		Model 2[‡]		Model 3[§]	
Quintiles of sex steroid hormones	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**
Total testosterone, ng/mL						
≤3.30	2.47	2.02 – 3.03	1.90	1.58 – 2.28	2.18	1.75 – 2.72
3.31–4.33	1.81	1.51 – 2.17	1.68	1.40 – 2.01	1.77	1.49 – 2.11
4.34–5.35	1.63	1.32 – 2.02	1.66	1.36 – 2.03	1.67	1.38 – 2.03
5.36–6.51	1.11	0.87 – 1.41	1.22	0.97 – 1.54	1.17	0.95 – 1.44
>6.51	1.04	0.87 – 1.24	1.26	1.04 – 1.52	1.08	0.85 – 1.39
Slope, P-trend[*]	−0.22	<0.001	−0.11	0.009	−0.18	0.001
Calculated free testosterone, ng/mL						
≤0.058	2.37	1.76 – 3.19	1.78	1.31 – 2.41	2.03	1.49 – 2.78
0.059–0.081	1.73	1.39 – 2.15	1.46	1.19 – 1.80	1.61	1.28 – 2.02
0.082–0.103	1.62	1.35 – 1.94	1.66	1.42 – 1.95	1.74	1.49 – 2.04
0.104–0.132	1.29	1.08 – 1.54	1.40	1.18 – 1.67	1.35	1.13 – 1.60
>0.132	1.14	0.88 – 1.48	1.39	1.10 – 1.77	1.17	0.91 – 1.51
Slope, P-trend[*]	−0.17	0.001	−0.05	0.27	−0.13	0.03
Total estradiol, pg/mL						
≤18.9	1.20	1.01 – 1.44	1.35	1.16 – 1.59	1.16	1.01 – 1.35
19.0–26.1	1.39	1.15 – 1.68	1.41	1.19 – 1.67	1.33	1.12 – 1.59
26.2–32.6	1.59	1.29 – 1.98	1.50	1.24 – 1.82	1.46	1.22 – 1.76
32.7–41.4	1.55	1.26 – 1.92	1.58	1.33 – 1.88	1.69	1.42 – 2.02
>41.4	1.94	1.65 – 2.29	1.78	1.52 – 2.08	2.09	1.73 – 2.52
Slope, P-trend[*]	0.11	0.001	0.07	0.05	0.14	<0.001
Calculated free estradiol, pg/mL						
≤0.058	1.11	0.90 – 1.38	1.39	1.17 – 1.66	1.23	1.02 – 1.48
0.059–0.080	1.27	1.02 – 1.57	1.29	1.04 – 1.60	1.19	0.98 – 1.45
0.081–0.104	1.44	1.12 – 1.84	1.43	1.20 – 1.71	1.39	1.17 – 1.66
0.105–0.133	1.72	1.39 – 2.14	1.58	1.32 – 1.91	1.63	1.35 – 1.95

	Model 1†		Model 2‡		Model 3§	
Quintiles of sex steroid hormones	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**
>0.133	2.18	1.85 – 2.56	1.94	1.66 – 2.27	2.33	1.90 – 2.85
Slope, P-trend*	0.17	<0.001	0.09	0.007	0.15	<0.001
Total testosterone to estradiol molar ratio						
≤0.104	2.93	2.44 – 3.52	2.27	1.90 – 2.70	2.24	1.85 – 2.72
0.105–0.138	1.82	1.54 – 2.15	1.69	1.43 – 2.01	1.68	1.41 – 2.01
0.139–0.174	1.46	1.18 – 1.80	1.47	1.20 – 1.80	1.47	1.21 – 1.79
0.175–0.231	1.20	1.00 – 1.44	1.27	1.09 – 1.47	1.27	1.09 – 1.49
>0.231	0.93	0.77 – 1.13	1.19	0.98 – 1.44	1.20	0.97 – 1.47
Slope, P-trend*	−0.27	<0.001	−0.16	<0.001	−0.15	<0.001
SHBG, nmol/L						
≤22.5	2.04	1.68 – 2.48	1.73	1.50 – 1.99	1.43	1.21 – 1.69
22.6–30.2	1.88	1.49 – 2.37	1.74	1.41 – 2.16	1.63	1.33 – 2.01
30.3–39.9	1.29	1.05 – 1.59	1.35	1.14 – 1.59	1.39	1.17 – 1.64
40.0–52.9	1.28	1.02 – 1.60	1.39	1.14 – 1.71	1.59	1.28 – 1.96
>52.9	1.09	0.83 – 1.42	1.35	1.06 – 1.72	1.67	1.29 – 2.15
Slope, P-trend*	−0.17	<0.001	−0.08	0.04	0.02	0.59
Androstanediol glucuronide, ng/mL						
≤4.70	1.26	0.99 – 1.61	1.28	1.06 – 1.53	Not applicable	
4.71–6.03	1.38	1.18 – 1.61	1.51	1.32 – 1.73		
6.04–7.41	1.82	1.46 – 2.25	1.80	1.43 – 2.27		
7.42–10.1	1.54	1.29 – 1.86	1.48	1.26 – 1.72		
>10.1	1.61	1.29 – 2.00	1.55	1.27 – 1.90		
Slope, P-trend*	0.06	0.11	0.03	0.30		

Abbreviations: SHBG, sex hormone binding globulin; CI, confidence interval

† From a linear regression model of sex steroid hormones on C-reactive protein adjusted for age and race/ethnicity

‡ Same as model 1 plus adjustment for body mass index, waist circumference, cigarette smoking, diabetes, alcohol consumption, and leisure-time physical activity

§ Same as model 2 plus testosterone, estradiol, and SHBG mutually adjusted, and calculated free testosterone and free estradiol mutually adjusted

* Per 1 weighted quintile change in sex steroid hormones

Table 3

Odds ratios and 95% confidence intervals of elevated C-reactive protein (≥3 vs. <3 mg/L) concentrations by quintiles of sex steroid hormones the U.S. population of adult men 20 years or older, NHANES 1999–2004

	Model 3[†]		
Quintiles of sex steroid hormones	**Odds ratio**	**95% CI**	**P-trend[‡]**
Total testosterone, ng/mL			
≤3.30	1.00 (ref)		
3.31–4.33	0.56	0.31 – 1.01	
4.34–5.35	0.71	0.40 – 1.26	
5.36–6.51	0.31	0.15 – 0.63	
>6.51	0.17	0.07 – 0.45	<0.001
Calculated free testosterone, ng/mL			
≤0.058	1.00 (ref)		
0.059–0.081	0.43	0.22 – 0.84	
0.082–0.103	0.56	0.34 – 0.94	
0.104–0.132	0.26	0.13 – 0.52	
>0.132	0.27	0.11 – 0.66	0.003
Total estradiol, pg/mL			
≤18.9	1.00 (ref)		
19.0–26.1	1.55	0.73 – 3.26	
26.2–32.6	2.22	1.14 – 4.33	
32.7–41.4	2.62	1.30 – 5.26	
>41.4	2.94	1.39 – 6.22	0.003
Calculated free estradiol, pg/mL			
≤0.058	1.00 (ref)		
0.059–0.080	1.17	0.59 – 2.32	
0.081–0.104	1.66	0.88 – 3.12	
0.105–0.133	1.95	0.96 – 3.95	
>0.133	3.41	1.55 – 7.48	0.001
Total testosterone to estradiol molar ratio			
≤0.104	1.00 (ref)		
0.105–0.138	0.77	0.46 – 1.31	
0.139–0.174	0.54	0.28 – 1.04	
0.175–0.231	0.45	0.22 – 0.92	
>0.231	0.32	0.14 – 0.72	0.005
SHBG, nmol/L			
≤22.5	1.00 (ref)		
22.6–30.2	1.27	0.67 – 2.39	
30.3–39.9	1.20	0.58 – 2.49	
40.0–52.9	1.82	0.79 – 4.23	
>52.9	1.79	0.70 – 4.57	0.18
Androstanediol glucuronide, ng/mL			

	Model 3†		
Quintiles of sex steroid hormones	**Odds ratio**	**95% CI**	**P-trend‡**
≤4.70	1.00 (ref)		
4.71–6.03	0.93	0.47 – 1.84	
6.04–7.41	1.56	0.78 – 3.12	
7.42–10.1	1.42	0.76 – 2.68	
>10.1	0.98	0.44 – 2.16	0.68

Abbreviations: SHBG, sex hormone binding globulin; CI, confidence interval

†From a logistic regression model of sex steroid hormones on elevated C-reactive protein (≥3 vs. <3 mg/L) adjusted for age, race/ethnicity, body mass index, waist circumference, cigarette smoking, diabetes, alcohol consumption, and leisure-time physical activity. Models of testosterone, estradiol, and SHBG are mutually adjusted, and models of calculated free testosterone and free estradiol are mutually adjusted

‡Per 1 weighted quintile change in sex steroid hormones

Table 4

Geometric means and 95% confidence intervals of white blood cell count (·10^9 cells/L) concentrations by quintiles of sex steroid hormones the U.S. population of adult men 20 years or older, NHANES 1999–2004

	Model 1†		Model 2‡		Model 3§	
Quintiles of sex steroid hormones	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**
Total testosterone, ng/mL						
≤3.30	6.49	6.23 – 6.76	6.40	6.11 – 6.71	6.44	6.06 – 6.85
3.31–4.33	6.68	6.41 – 6.96	6.68	6.43 – 6.93	6.68	6.43 – 6.95
4.34–5.35	6.35	6.09 – 6.63	6.46	6.17 – 6.75	6.44	6.17 – 6.74
5.36–6.51	6.49	6.20 – 6.80	6.51	6.22 – 6.82	6.52	6.22 – 6.85
>6.51	6.36	5.98 – 6.77	6.30	5.99 – 6.63	6.26	5.91 – 6.62
Slope, P-trend *	−0.007	0.39	−0.007	0.40	−0.009	0.45
Calculated free testosterone, ng/mL						
≤0.058	6.45	6.12 – 6.79	6.30	5.95 – 6.66	6.45	6.08 – 6.83
0.059–0.081	6.38	6.08 – 6.68	6.31	6.00 – 6.62	6.41	6.11 – 6.72
0.082–0.103	6.45	6.25 – 6.67	6.51	6.27 – 6.75	6.56	6.31 – 6.81
0.104–0.132	6.41	6.12 – 6.72	6.48	6.16 – 6.81	6.43	6.13 – 6.75
>0.132	6.66	6.26 – 7.08	6.69	6.35 – 7.05	6.49	6.15 – 6.85
Slope, P-trend *	0.008	0.52	0.01	0.21	0.001	0.91
Total estradiol, pg/mL						
≤18.9	6.11	5.90 – 6.32	6.30	6.09 – 6.50	6.22	6.00 – 6.46
19.0–26.1	6.42	6.09 – 6.77	6.42	6.09 – 6.77	6.43	6.09 – 6.78
26.2–32.6	6.50	6.27 – 6.74	6.48	6.25 – 6.72	6.49	6.26 – 6.73
32.7–41.4	6.45	6.19 – 6.73	6.44	6.21 – 6.67	6.48	6.24 – 6.72
>41.4	6.90	6.49 – 7.34	6.73	6.35 – 7.14	6.75	6.34 – 7.19
Slope, P-trend *	0.02	0.004	0.01	0.09	0.02	0.08
Calculated free estradiol, pg/mL						
≤0.058	6.03	5.85 – 6.21	6.26	6.07 – 6.44	6.25	6.05 – 6.46
0.059–0.080	6.33	6.01 – 6.67	6.37	6.02 – 6.74	6.37	6.02 – 6.75
0.081–0.104	6.44	6.18 – 6.70	6.34	6.11 – 6.57	6.34	6.11 – 6.57

	Model 1†		Model 2‡		Model 3§	
Quintiles of sex steroid hormones	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**
0.105–0.133	6.52	6.30 – 6.75	6.50	6.28 – 6.73	6.50	6.28 – 6.73
>0.133	7.02	6.61 – 7.46	6.88	6.49 – 7.29	6.89	6.49 – 7.31
Slope, P-trend*	0.03	<0.001	0.02	0.01	0.02	0.02
Total testosterone to estradiol molar ratio						
≤0.104	6.91	6.49 – 7.35	6.80	6.45 – 7.18	6.74	6.39 – 7.11
0.105–0.138	6.62	6.36 – 6.89	6.58	6.29 – 6.89	6.56	6.26 – 6.87
0.139–0.174	6.41	6.14 – 6.69	6.41	6.16 – 6.68	6.43	6.18 – 6.69
0.175–0.231	6.50	6.20 – 6.82	6.48	6.21 – 6.75	6.50	6.24 – 6.77
>0.231	6.01	5.81 – 6.22	6.15	5.94 – 6.37	6.18	5.95 – 6.42
Slope, P-trend*	−0.03	0.001	−0.02	0.01	−0.02	0.04
SHBG, nmol/L						
≤22.5	6.69	6.34 – 7.07	6.80	6.45 – 7.17	6.75	6.39 – 7.14
22.6–30.2	6.66	6.39 – 6.95	6.67	6.37 – 6.98	6.67	6.37 – 6.99
30.3–39.9	6.29	6.01 – 6.59	6.35	6.10 – 6.61	6.37	6.11 – 6.64
40.0–52.9	6.37	6.05 – 6.71	6.29	6.01 – 6.59	6.31	6.01 – 6.63
>52.9	6.26	5.90 – 6.64	6.10	5.77 – 6.44	6.10	5.70 – 6.53
Slope, P-trend*	−0.02	0.04	−0.03	0.005	−0.03	0.04
Androstanediol glucuronide, ng/mL						
≤4.70	6.50	6.18 – 6.82	6.34	6.09 – 6.60	Not applicable	
4.71–6.03	6.47	6.17 – 6.79	6.51	6.22 – 6.82		
6.04–7.41	6.59	6.23 – 6.98	6.57	6.23 – 6.94		
7.42–10.1	6.42	6.17 – 6.68	6.42	6.16 – 6.68		
>10.1	6.41	6.06 – 6.78	6.52	6.19 – 6.86		
Slope, P-trend*	−0.004	0.65	0.003	0.63		

Abbreviations: SHBG, sex hormone binding globulin; CI, confidence interval

†From a linear regression model of sex steroid hormones on white blood cell count adjusted for age and race/ethnicity

‡Same as model 1 plus adjustment for body mass index, waist circumference, cigarette smoking, diabetes, alcohol consumption, and leisure-time physical activity

§Same as model 2 plus testosterone, estradiol, and SHBG mutually adjusted, and calculated free testosterone and free estradiol mutually adjusted

* Per 1 weighted quintile change in sex steroid hormones