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Trends in sex hormone concentrations in U.S. males: 1988–1991 to 1999–2004

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Abstract

Previous studies suggest that male testosterone concentrations have declined over time. To explore this in a large US population, we examined testosterone and free testosterone concentrations in National Health and Nutrition Examination Surveys (NHANES) from 1988–1991 and 1999–2004. We also examined sex hormone-binding globulin (SHBG), estradiol, and androstanediol glucuronide (3α-diol-G) over the same period. Non-Hispanic white, non-Hispanic black, and Mexican-American men from 1988–1991 and 1999–2004 NHANES surveys who were ≥ 20 years old and had serum from morning blood draws were included in this analysis (1988–1991: N=1,413; 1999–2004: N=902). Testosterone, estradiol, and SHBG were measured by competitive electrochemiluminescence immunoassays and 3α-diol-G was measured by enzyme immunoassay. Free testosterone was calculated using testosterone and SHBG values. Adjusted mean hormone concentrations were estimated using linear regression, accounting for NHANES sampling weights and design, age, race/ethnicity, body mass index, waist circumference, alcohol use, and smoking. Differences in adjusted mean concentrations (Δ) and two-sided P-values were calculated; $P<0.05$ was statistically significant. Overall, 3α-diol-G and estradiol declined between 1988–1991 and 1999–2004, but there was little change in testosterone, free testosterone, or SHBG (Δ: 3α-diol-G = −1.83 ng/mL, $P<0.01$; estradiol=−6.07 pg/mL, $P<0.01$; testosterone=− 0.03 ng/mL, P=0.75; free testosterone=−0.001 ng/mL, P=0.67; SHBG=−1.17 nmol/L, P=0.19). Stratification by age and race revealed that SHBG and 3α-diol-G declined among whites 20–44 years old (Δ: SHBG=−5.14 nmol/L, $P<0.01$; 3α-diol-G =−2.89 ng/mL, $P<0.01$) and free testosterone increased among blacks 20–44 years old (Δ: 0.014, P=0.03). Estradiol declined among all ages of whites and Mexican-

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KAM, EAP, GMM and SR contributed to the study design. GB performed the laboratory assays. SJN, BIG, and KAM analyzed and interpreted the data, and drafted the manuscript. YL, GMM, EAP, SR, and GB provided comments and revisions to the manuscript. SJN, BIG, YL, GMM, EAP, SR, GB, and KAM reviewed and approved the final manuscript.

Americans. In conclusion, there was no evidence for testosterone decline between 1988–1991 and 1999–2004 in the US general population. Subgroup analyses suggest that SHBG and 3α-diol-G declined in young white men, estradiol declined in white and Mexican-American men, and free testosterone increased in young black men. These changes may be related to the increasing prevalence of reproductive disorders in young men.

Key terms

testosterone; androstanediol glucuronide; estradiol; sex hormone-binding globulin; epidemiology; National Health and Nutrition Examination Survey

INTRODUCTION

Reports in the literature suggest that there have been declines in male reproductive health, including lower sperm quality and higher rates of cryptorchidism, hypospadias, and testicular germ cell tumors (TGCTs), collectively referred to as testicular dysgenesis syndrome (TDS) (James 2010; Skakkebaek et al. 2001). It has been hypothesized that TDS may involve reduced testosterone concentrations, due to malformation and improper functioning of Leydig cells in the testes (Skakkebaek et al. 2001). Murine models have reported that *in utero* exposure of male fetuses to chemicals that produce the physiologic hallmarks of TDS results in lower testicular testosterone concentrations in the fetus, but normal plasma testosterone concentrations in adulthood (Fisher et al. 2003; Mahood et al. 2005; Mahood et al. 2007). Whether a link exists between testosterone concentrations and reproductive abnormalities in humans is unclear. Nor has it been established whether population testosterone concentrations in humans have declined.

Despite the increasing interest in secular declines in male hormone concentrations, only two epidemiological studies have evaluated changes in male testosterone concentrations over time. Among Danish men who participated in studies between 1982 and 2001, there was no significant change in testosterone concentration over time after adjustment for body mass index (BMI) (Andersson et al. 2007). Higher BMI is associated with lower testosterone concentrations (Macdonald et al. 2010), and population increases in BMI may have been an underlying cause of the decline in testosterone. However, a study of US men, the Massachusetts Male Aging Study (MMAS), reported that testosterone declined among men followed from 1987–1989 to 2002–2004, even after adjustment for BMI and other factors (Travison et al. 2007).

The question of whether male testosterone levels have declined over time remains unanswered due to the conflicting results from Andersson et al. (Andersson et al. 2007) and Travison et al. (Travison et al. 2007). Additionally, the population studied by Travison et al. was drawn from a narrowly-defined geographic area, and it is not clear if the results can be extrapolated to the broader U.S. population. Therefore, we compared concentrations of testosterone and free testosterone in men from the 1988–1991 and 1999–2004 National Health and Nutrition Examination Surveys (NHANES), nationally representative cross-sectional studies in the US. We also examined concentrations of estradiol, sex hormone-binding globulin (SHBG), and androstanediol glucuronide (3α-diol-G). SHBG is a carrier

protein that binds testosterone and estradiol, and 3α-diol-G, a metabolite of dihydrotestosterone (DHT), is correlated with tissue androgen concentrations. TGCTs occur predominantly among men 20–44 years old and are more common among white than non-white men (Bray et al. 2006; Mcglynn et al. 2003), leading us to hypothesize that if changes in male hormones were related to TDS, these changes might be most evident in young white men. To explore this hypothesis, we examined changes in hormones by race/ethnicity and age.

MATERIALS AND METHODS

Study Population

National Health and Nutrition Examination Survey—NHANES is a series of cross-sectional surveys conducted by the Centers for Disease Control and Prevention (CDC) in order to assess the health and nutrition status of Americans (Http://Www.Cdc.Gov/Nchs/Nhanes.Htm). Participant sampling follows a complex stratified, multistage, probability-cluster design in order to select a representative sample of the civilian, non-institutionalized US population based on age, gender, and race/ethnicity. Participants are not followed longitudinally. Therefore, participants in the 1999–2004 survey were not the same individuals as the participants in the 1988–1991 survey. The survey interview includes demographic, socioeconomic, and health-related questions. The examination component consists of laboratory tests, medical and physiological measurements, and blood collection. Once routine testing of blood samples is completed, the CDC allows investigators to access surplus sera for approved scientific studies.

In this study, we estimated hormone concentrations for non-Hispanic white (hereafter referred to as white), non-Hispanic black (hereafter referred to as black), and Mexican-American men only. Other racial/ethnic groups were excluded due to small sample sizes.

Protocols for the 1988–1991 and 1999–2004 NHANES surveys were conducted in accordance with the Declaration of Helsinki and were approved by the Ethics Review Board of the National Center for Health Statistics (NCHS), U.S. Centers for Disease Control and Prevention. Documented informed consent was obtained from all participants. Measurement of sex steroid hormones was approved by the National Institutes of Health Office of Human Subjects Research and the National Center for Health Statistics Ethics Review Board.

NHANES 1988–1991—Characteristics of the 1988–1991 survey participants and their mean hormone concentrations were reported previously (Rohrmann et al. 2007). Briefly, there were 4,227 male participants who were at least 20 years old at the time of interview. 4,122 of these men participated in the examination, of whom 2,009 participated in the morning examination. Of these men, 1,470 had surplus serum samples available for analysis. The 1988–1991 analytic population included the 1,413 participants who were white (N=674), black (N=363), or Mexican American (N=376) (supplementary figure).

NHANES 1999–2004—There were 15,184 male participants in the 1999–2004 surveys; 7,223 were aged 20 years or older. A total of 6,735 of these men participated in the examination, of whom 3,281 participated in the morning examination. Hormone

concentrations were examined within a onethird, stratified random sample of participants that had already been selected for a study of serum organochlorine concentrations. A total of 1,085 men who attended the morning exam were in this subsample, of whom 985 had surplus serum available for analysis. The 1999–2004 analytic population included the 902 men who were white (N=503), black (N=183) or Mexican American (N=216) (supplementary figure).

Hormone Measurement

Serum was separated from blood obtained during the NHANES examination and stored at −80°C. For 1988–1991 survey participants, testosterone, estradiol, and SHBG were measured using the Elecsys 2010 system (Roche Diagnostics, Laval, QC, Canada) and 3α-diol-G was measured using the DSL-10–9200 ACTIVE® Androstanediol Glucuronide EIA kit (Diagnostic Systems Laboratories, Webster, TX) in the laboratory of Dr. Nader Rifai (Children's Hospital, Boston, MA). Laboratory methods and quality control statistics were reported previously (Rohrmann et al. 2007).

Serum testosterone, estradiol, 3α-diol-G, and SHBG concentrations for 1999–2004 survey participants were measured in the same laboratory. Testosterone, estradiol, and SHBG were measured using the Elecsys 2010 system. 3α-diol-G was measured by enzyme immunoassay using the Direct 3α Diol-G ELISA kit (ALPCO Diagnostics, Salem, NH). A subset of 21 1999–2004 samples was assayed in duplicate to assess reproducibility. Coefficients of variation (CVs) were 4.8% for testosterone, 5.6% for SHBG, 9.7% for 3α-diol-G, and 21.4% for estradiol.

To examine the comparability of assays conducted for the two analysis populations, serum from 1988–1991 participants was retrieved from storage and re-assayed at the same time as the 1999–2004 samples. CVs for 1988–1991 samples assayed at both time points were 6.3% for testosterone, 5.3% for SHBG, 35.0% for 3α-diol-G, and 15.3% for estradiol. The higher CV for 3α-diol-G was likely due to the change in assay kit. The DSL-10–9200 EIA kit, used for samples from the 1988–1991 survey, was discontinued; 1999–2004 samples were assayed using the Direct 3α-diol-G ELISA kit.

3α-diol-G concentrations were on average 21% lower when measured with the Direct 3α-diol-G ELISA kit compared to the DSL-10–9200 EIA kit. Using the repeated sample data, a regression equation was formulated to account for the shift in values, similar to methods used for the analysis of vitamin D concentrations in NHANES (National Center for Health Statistics, 2010). All 3α-diol-G concentrations for 1999–2004 participants were adjusted using this equation:

$$3\alpha\text{-diol-G}_{1999\text{–}2004\ \text{adjusted}} = 0.68579 + 1.30589 * 3\alpha\text{-diol-G}_{1999\text{–}2004\ \text{measured}}$$

Free testosterone was calculated using the testosterone and SHBG values, according to the method described by Vermeulen et al. (Vermeulen et al. 1999).

Statistical Analysis

Covariates—Age, race/ethnicity, cigarette smoking, and alcohol use were self-reported in the NHANES interviews. Never smokers were defined as men who smoked fewer than 100

cigarettes during their lifetimes. Former smokers were defined as men who smoked at least 100 cigarettes in their lifetimes but now smoked “not at all”. Current smokers were defined as men who smoked at least 100 cigarettes in their lifetimes and currently smoked “every day” or “some days”. Men who drank fewer than 12 drinks annually were classified as having 0 drinks per week. Waist circumference, height, and weight were measured during the examination, and BMI (kg/m^2) was calculated from height and weight. Percent body fat was calculated from height, weight, and bioelectrical impedance analysis (BIA) (Chumlea et al. 2002), however, in 1999–2004 BIA was performed only on participants younger than 50 years old who weighed less than 300 pounds. Physical activity was not included as a covariate due to differences in the way variables were coded in the 1988–1991 and 1999–2004 surveys.

Analysis—Mean hormone concentrations and 95% confidence intervals (CIs) were estimated using linear regression, accounting for NHANES sampling weights and the complex sample design. Estradiol, 3α-diol-G, SHBG, and free testosterone concentrations were log-transformed because their sample distributions were skewed; geometric means were estimated and back-transformed for presentation. The distribution of testosterone was approximately normal, thus testosterone concentrations were not transformed for analysis.

Adjusted means were estimated using predicted margins with several linear regression models. First, mean hormone concentrations were adjusted for the age (1-year intervals) and race/ethnicity distribution of the combined (1988–1991 and 1999–2004) population. In the second model, means were adjusted for age, race/ethnicity, BMI, waist circumference, alcohol use, and smoking.

All regression models incorporated variables indicating sampling weights, stratification and clustering. Although the number of men included in the analysis is a small proportion of the total number of males interviewed for each NHANES survey, the analytic sample was derived from a series of random subsamples and is representative of the larger survey population. Sampling weights are specifically designed by the NCHS to account for this sampling process such that subsample estimates using the sampling weights are representative of the estimates that would have been obtained from larger NHANES survey population. Thus, mean hormone concentrations reflect population-level estimates.

Sensitivity analyses were conducted to investigate the effect of adjusting for additional factors that may have influenced hormone concentrations. To determine the effect of adjusting for percent body fat instead of BMI, we estimated mean hormone concentrations adjusted for age, race/ethnicity, waist circumference, alcohol use, smoking and either percent body fat or BMI among men younger than 50 years old. To examine the influence of BMI outliers, we estimated change in hormone concentrations after excluding men with BMI in the lowest and highest 5% of the distribution; the lowest and highest 5% was determined separately for each race/ethnicity group. To determine the effect of co-morbidities on mean hormone concentrations, models were additionally adjusted for self-reported history of arthritis, stroke, diabetes, osteoporosis, heart attack, heart failure and general health status. Variables were added individually to a regression model that adjusted for age, race/ethnicity, waist circumference, alcohol use, smoking and BMI. To evaluate

whether the use of medications affected the difference in hormone concentrations between the two survey populations, we estimated mean hormone concentrations using regression models that additionally adjusted for recent use of selected medications. During the NHANES interview, men were asked if they had taken any medications in the past month for which a doctor's or dentist's prescription was needed; if they answered yes, they were asked the name of the medication. We identified the following medications that may affect sex hormone levels from the NHANES prescription drug file: finasteride, testosterone, flutamide, atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, and simvastatin. Overall, there were 14 individuals in the 1988–1991 analytic population and 52 men in the 1999–2004 analytic population who reported taking at least one of these medications in the month before the survey.

Differences in mean hormone concentrations were calculated by subtracting the 1988–1991 means from the 1999–2004 means. Two-sided P-values for differences in means were calculated from ANOVA analyses; P-values less than 0.05 were considered statistically significant. Analyses were conducted using SUDAAN, v10.0 (RTI International, Research Triangle Park, NC) and SAS v9.1 (SAS, Cary, NC).

RESULTS

Participant characteristics

The 1,413 1988–1991 NHANES participants, had a median age of 46 years and 49% were white (table 1). The median BMI among the participants was 25.8 kg/m^2, the median waist size was 94.5 cm, and the median body fat percentage was 25.9. Most men (70%) were not current smokers and 38% did not drink alcohol.

The 902 1999–2004 NHANES participants had a median age of 48 years and 57% were white (table 1). The median BMI was 27.3 kg/m^2 and the median percentage of body fat among men younger than 50 years old was 24.6%. Median waist size was smallest among participants aged 20–44 years (94 cm) and largest among participants aged 45–69 years (103 cm). Younger participants were more commonly never-smokers and older participants were more commonly former smokers. Thirty percent of participants abstained from alcohol.

Differences in hormone concentrations between 1988–1991 and 1999–2004

Differences in mean hormone concentrations were first estimated adjusted only for age and race/ethnicity. These minimally adjusted models showed significant declines in mean concentrations of testosterone, free testosterone, SHBG, 3α-diol-G, and estradiol between 1988–1991 and 1999–2004 (testosterone, P<0.01; free testosterone, P=0.02; SHBG, P<0.01; 3α-diol-G, P<0.01; estradiol, P<0.01; supplementary table 1). However, when models were fully adjusted for the additional covariates BMI, waist size, smoking, and alcohol consumption, there were no significant differences in testosterone, free testosterone, or SHBG concentrations (all P>0.05, table 2). The fully adjusted models continued to show significant declines in mean 3α-diol-G and estradiol (both P < 0.01; table 2).

Stratification by age suggested that changes in some hormone concentrations occurred mainly in younger men. There were significant declines in SHBG (P<0.01) and 3α-diol-G

(P<0.01) among men 20–44 years old, but not among men 45 and older (table 3). In contrast, SHBG concentrations increased in men 45–69 years old (P=0.04) and estradiol declined in men of all ages (table 3). There were no changes in testosterone or free testosterone for any age group.

Differences in hormone concentrations also varied by race/ethnicity. Mean 3α-diol-G and estradiol concentrations declined significantly among white and Mexican-American men (3α-diol-G, white P<0.01, Mexican-American P=0.03; estradiol, white P<0.01, Mexican-American P<0.01), but not black men (table 4). On the contrary, testosterone (P=0.02) and free testosterone (P=0.04) concentrations increased among black men, but not among white or Mexican-American men (table 4).

Cross-stratification by age and race/ethnicity further suggested that changes in hormone profiles varied in the population. Significant declines in SHBG and 3α-diol-G were limited to 20–44 year-old white men (SHBG P<0.01; 3α-diol-G P<0.01; table 5). Though concentrations of 3α-diol-G tended to be lower in 1999–2004 among all age groups of Mexican-American men, the differences were not statistically significant after age stratification. There were significant declines in estradiol in all age groups of white and Mexican-American men, but not among black men of any age (table 5). The increase in free testosterone concentration that was observed among black men was restricted to the youngest and oldest age groups (table 5). Testosterone levels also appeared higher among black men 20–44 and ≥ 70 years old, but did not reach statistical significance.

Sensitivity analyses

We explored whether the observed changes in hormone concentrations were due to changes in the prevalence of obesity. Differences in hormone concentrations among young men were similar for models that adjusted for percent body fat rather than BMI (data not shown). When we restricted the analyses to young white men with BMI between the 5^{th} and 95^{th} percentiles (20.4 to 36.6 kg/m^2) the magnitude of decline for testosterone and SHBG diminished; the decline in SHBG was no longer statistically significant, but declines in 3α-diol-G and estradiol were still present (supplementary table 5). We also examined whether increases in testosterone and free testosterone in black men were affected by extreme BMIs. Among black men with BMIs between the 5^{th} and 95^{th} percentiles (19.2 to 37.6 kg/m^2), increases in testosterone and free testosterone were essentially unchanged from the what was observed in the full population (supplementary table 6). Additional adjustment for co-morbidities, general health, or medication use did not change the results for comparisons in the overall population or within age or race/ethnicity-stratified groups (data not shown).

DISCUSSION

Our examination of sex hormone concentrations among US males during 1988–1991 and 1999–2004 found that after adjustment for covariates there were declines in 3α-diol-G and estradiol, but not testosterone, free testosterone, or SHBG. Stratified analyses revealed declines in 3α-diol-G and SHBG concentrations among 20–44 year old white men. Although testosterone was lower among young white men, the decline was not significant, and sensitivity analyses showed that much of the difference was driven by men with extreme

BMIs. Though there was no change in testosterone or free testosterone the overall population, we found that there were increases in both among black men. Comparisons among this subgroup were limited by small sample size, especially when also stratified by age, but the results were robust to the influence of extreme BMIs in the population. Replication in another population is needed to confirm this result, but these data suggest that changes in hormones over time vary by age and race/ethnicity group.

The lack of decline in testosterone and free testosterone in the NHANES population is in agreement with the results of Andersson et al. (Andersson et al. 2007), who found no decline in testosterone or free testosterone in Danish men after adjusting for BMI, suggesting that the decline they observed in unadjusted analyses was confounded by changes in body size. Our results do not agree with those from Travison et al. (Travison et al. 2007), which reported testosterone declines in unadjusted and adjusted analyses.

Reasons for the discrepancy between our study and that of Travison et al. (Travison et al. 2007) are not clear. The study populations were similar in terms of calendar time - NHANES and MMAS surveys were both conducted between the late 1980s to early 2000s, so differences between the two are not due to analysis of change in hormones concentrations over different lengths of time. Furthermore, although the MMAS involved predominantly white men and NHANES included multiple racial groups, there was still no decline in testosterone when NHANES analyses were limited to white men.

One potential reason for the difference between these two US studies could be a difference in the prevalence of confounders. Factors such as age, body size, smoking, alcohol use, and chronic disease are associated with serum hormone concentrations in males (Akishita et al. 2010; Allen et al. 2002; Andersson et al. 2007; Dai et al. 1988; Derby et al. 2006; Field et al. 1994; Gapstur et al. 2002; Gapstur et al. 2007; Haring et al. 2010; Harman et al. 2001; Hautanen et al. 1994; Lapauw et al. 2008; Liu et al. 2007; Macdonald et al. 2010; Svartberg et al. 2004; Travison et al. 2007; Wu et al. 1995; Yeap et al. 2009), and previous reports of declines in testosterone may be related to changing distributions of these factors. In NHANES, the magnitudes of decline in testosterone and SHBG in young white men were both diminished after excluding men with extreme BMIs, demonstrating that extreme BMIs of the magnitude seen in the general US population are prevalent enough to influence estimates of the population mean testosterone and SHBG concentrations. It is possible that the difference in results between studies is due to the presence of men with extremely high BMIs in some study populations and not others. The two studies may also have been influenced by unknown confounders. Differences between NHANES and MMAS in how the prevalence of an unknown confounder changed over time could have resulted in different amounts of confounding bias in the two studies, resulting in a significant decline in testosterone over time in the MMAS and no significant change in NHANES.

There were significant declines in 3α-diol-G and estradiol in white and Mexican-American men. Population trends in 3α-diol-G and estradiol have not been studied as extensively as testosterone, and to our knowledge there have been no other studies of population trends in estradiol or 3α-diol-G concentrations in adult men. Therefore, confirmation of these results in other studies is necessary to determine the consistency of these trends in other

populations. Estradiol and 3α-diol-G are both produced from testosterone by enzymatic conversion. That the strong declines in these hormones were seen in the absence of a decline in testosterone suggests that the declines are the result of a change in factors affecting the testosterone metabolism process. If the estradiol and 3α-diol-G declines are confirmed in other populations, analysis of temporal changes in other androgens may help pinpoint which parts of the testosterone metabolism pathway are affected.

Like testosterone, 3α-diol-G and estradiol are associated with anthropometric and lifestyle factors. 3α-diol-G concentrations decrease with age and HDL cholesterol (Allen et al. 2002; Hautanen et al. 1994; Suzuki et al. 2009), and increase with increasing BMI, alcohol use, body fat, and visceral adipose tissue (Allen et al. 2002; Tchernof et al. 1997; Wu et al. 2001). Estradiol concentrations tend to increase among elderly men (Abbott et al. 2007; Haffner et al. 1993). Higher estradiol concentrations in middle aged and older men have been associated with increased risk of stroke, atrial fibrillation, dementia, and all-cause mortality (Abbott et al. 2007; Szulc et al. 2009). The current study was able to control for the effects of some but not all of the factors associated with 3α-diol-G and estradiol concentrations; declines in estradiol and 3α-diol-G persisted after adjustment and after excluding men with extreme BMIs.

Serum samples from the two NHANES surveys were collected and measured at different times, and comparisons may be susceptible to bias from evaporation, assay variation, or other unknown factors. We took steps to minimize variation in how the samples were treated, including using the same laboratory and same assays to measure testosterone, estradiol, and SHBG. Unfortunately, production of the kit used to measure 3α-diol-G in 1988–1991 samples was discontinued before the 1999–2004 samples were measured. As a result, the 1999–2004 samples were measured using a different kit, which was likely responsible for the high variation among the 1988–1991 samples measured during the two lab sessions. The CV for 3α-diol-G in 1999–2004 samples analyzed in duplicate using the Direct 3α-diol-G kit was 9.7%, indicating that the assay itself had an acceptable level of variability. Due to the use of different assays kits for the two survey periods, a regression equation was formulated to account for the shift in values and to generate adjusted 3α-diol-G concentrations that were used in the statistical analysis. Results should be interpreted with the knowledge that although attempts were made to correct for the change in assay kits, there may be residual bias in the adjusted values.

Estradiol measurements displayed unusually high variation for both 1999–2004 population samples measured in duplicate and for 1988–1991 samples measured during both lab sessions, even though the same laboratory method was used for all measurements. The relatively low CVs for SHBG and testosterone suggest that variation in estradiol is likely due to assay-specific rather than serum-specific factors. It is unlikely that this increased variability is driving the decline in estradiol concentrations observed among whites and Mexican-Americans. Increased assay variability would create a larger spread in the distribution of individual hormone values around the mean, and the wider spread would result in more overlap between the distributions being compared making it less likely, not more likely, that the distributions would be statistically different. Furthermore, if the observed declines were an artifact of the assay we would expect to see similar declines in all

groups of men; however, no decline was observed among black men. Still, the results for estradiol should be interpreted with appropriate caution. Analyses in other populations are needed to provide a more accurate estimate of the magnitude of the decline.

NHANES populations are sampled to be representative of the civilian, non-institutionalized US population. Thus, these results are more generalizable to changes in male hormone concentrations that have occurred in the general US population than changes observed in individual studies conducted in narrowly-defined populations. The NHANES sampling design provided sufficient numbers of participants to estimate hormone concentrations stratified by race/ethnicity and age group. Rohrmann et al. (Rohrmann et al. 2007) previously reported that there were differences in concentrations of 3α-diol-G, estradiol, and SHBG by race/ethnicity in US men, so it was of interest to investigate changes in hormone concentrations within these groups as well as in the overall population. We found that some changes in hormone concentrations were similar for whites and Mexican-Americans when comparing 1988–1991 and 1999–2004 surveys, and patterns for blacks were often different from those for whites and Mexican-Americans. Moreover, factors that influence hormone concentrations, such as body size, smoking, and alcohol use, vary by demographic factors and geography. Additional analysis of demographic factors may be useful in identifying subpopulations in which hormone concentrations may be changing over time.

In conclusion, adjusted mean testosterone concentrations were similar between 1988–1991 and 1999–2004 in the overall US adult male population. Exploration of the relative importance of total testosterone and free testosterone in reproductive health is necessary, as free testosterone concentrations were stable in white and Mexican Americans, and may have increased in blacks. Adjusted concentrations of 3α-diol-G and estradiol declined in whites and Mexican-Americans, but the overall significance of these declines is unknown at this time. Examination of these trends and any association with male reproductive conditions in other populations would be beneficial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of 1988–1991 and 1999–2004 NHANES participants.

	1988–1991				1999–2004			
		Age at interview (years)				Age at interview (years)		
	All	**20–44**	**45–69**	**≥ 70**	**All**	**20–44**	**45–69**	**≥ 70**
N	1413	675	483	255	902	398	342	162
Median age, years	46	31	57	76	48	32	57	77
Median BMI, kg/m^2	25.8	25.4	26.8	25.8	27.3	26.5	28.5	26.7
Median waist circumference, cm	94.5	89.5	98.0	99.0	98.8	94.0	103.0	102.2
Median % bodyfat[a]	25.9	25.1	26.7	26.8	24.6	24.3	25.4	---
Race/ethnicity, N								
White	674	240	244	190	503	187	199	117
Black	363	202	121	40	183	99	66	18
Mexican-American	376	233	118	25	216	112	77	27
Cigarette smoking, %								
Never smoker	35	44	24	33	41	53	33	33
Former smoker	35	20	44	57	31	14	37	59
Current smoker	30	37	32	11	27	33	30	8
Number of alcohol drinks per week, %								
0	38	24	42	65	30	21	35	45
less than 1	8	10	7	7	22	23	21	23
1 to less than 7	28	34	25	15	27	37	21	15
7 or more	26	31	26	13	20	19	23	17

[a] 1999–2004 surveys: percent body fat only measured among those < 50 years old and weighing less than 300 lbs

Table 2

Mean hormone concentrations in adult US males, comparing NHANES 1988–1991 and 1999–2004.

	1988–1991		1999–2004			
	Mean[a, b]	95% CI[c]	Mean[a, b]	95% CI[c]	Difference	P-value
Testosterone (ng/ml)	5.37	5.20, 5.53	5.34	5.16, 5.52	−0.03	0.75
Free testosterone (ng/ml)	0.099	0.094, 0.103	0.097	0.093, 0.103	−0.001	0.67
SHBG (nmol/L)	34.71	33.36, 36.12	33.54	32.10, 35.04	−1.17	0.19
3α-diol-G (ng/ml)	11.98	11.41, 12.58	10.15	9.72, 10.60	−1.83	<0.01
Estradiol (pg/ml)	35.74	34.25, 37.29	29.67	28.20, 31.20	−6.07	<0.01

[a] adjusted for age (1 year intervals), race/ethnicity, BMI, waist circumference, smoking, and alcohol use

[b] means for free testosterone, SHBG, 3α-diol-G, and estradiol are geometric means

[c] CI – confidence interval

Table 3

Difference in mean hormone concentrations by age group, comparing NHANES 1988–1991 to 1999–2004.

		1988–1991		1999–2004			
	Age	Mean[a, b]	95% CI[c]	Mean[a, b]	95% CI[c]	Difference	P-value
Testosterone (ng/ml)	20–44	5.49	5.25, 5.73	5.29	5.03, 5.55	−0.20	0.15
	45–69	5.20	4.95, 5.45	5.38	5.10, 5.67	0.18	0.21
	≥ 70	5.15	4.64, 5.67	5.34	4.77, 5.91	0.19	0.39
Free testosterone (ng/ml)	20–44	0.102	0.096, 0.108	0.103	0.097, 0.109	0.001	0.67
	45–69	0.098	0.089, 0.109	0.095	0.088, 0.103	−0.003	0.55
	≥ 70	0.083	0.071, 0.097	0.073	0.060, 0.089	−0.010	0.19
SHBG (nmol/L)	20–44	35.75	33.38, 38.29	31.69	29.62, 33.90	−4.06	<0.01
	45–69	32.56	30.01, 35.32	35.19	32.17, 38.50	2.64	0.04
	≥ 70	35.54	30.07, 42.01	38.35	32.79, 44.85	2.81	0.13
3α-diol-G (ng/ml)	20–44	12.29	11.40, 13.26	9.80	9.13, 10.52	−2.49	<0.01
	45–69	11.38	10.14, 12.77	10.45	9.56, 11.43	−0.92	0.15
	≥ 70	12.09	9.85, 14.85	10.98	9.21, 13.08	−1.11	0.20
Estradiol (pg/ml)	20–44	35.53	33.70, 37.47	29.45	27.45, 31.60	−6.08	<0.01
	45–69	35.36	32.34, 38.67	29.95	27.74, 32.34	−5.41	<0.01
	≥ 70	38.61	34.23, 43.56	29.98	25.59, 35.13	−8.63	<0.01

[a] adjusted for age, race/ethnicity (1 year intervals), BMI, waist circumference, smoking, and alcohol use

[b] means for free testosterone, SHBG, 3α-diol-G, and estradiol are geometric means

[c] CI – confidence interval

Table 4

Difference in mean hormone concentrations by race/ethnicity, comparing NHANES 1988–1991 to 1999–2004.

	Race/ ethnicity	1988–1991		1999–2004			
		Mean[a, b]	95% CI[c]	Mean[a, b]	95% CI[c]	Difference	P-value
Testosterone (ng/ml)	White	5.38	5.20, 5.56	5.28	5.08, 5.49	−0.10	0.37
	Black	5.33	4.97, 5.69	5.90	5.55, 6.26	0.57	0.02
	Mexican-American	5.29	5.12, 5.46	5.09	4.76, 5.43	−0.20	0.26
Free testosterone (ng/ml)	White	0.099	0.094, 0.104	0.096	0.091, 0.102	−0.003	0.45
	Black	0.096	0.089, 0.104	0.107	0.098, 0.117	0.011	0.04
	Mexican-American	0.099	0.095, 0.104	0.095	0.088, 0.102	−0.005	0.23
SHBG (nmol/L)	White	34.87	33.37, 36.43	33.42	31.79, 35.13	−1.45	0.15
	Black	34.37	32.22, 36.66	34.47	31.72, 37.45	0.10	0.95
	Mexican-American	33.11	31.42, 34.88	33.24	30.75, 35.93	0.13	0.93
3α-diol-G (ng/ml)	White	12.22	11.55, 12.92	10.16	9.64, 10.71	−2.06	<0.01
	Black	10.90	10.02, 11.85	10.25	9.57, 10.99	−0.65	0.24
	Mexican-American	10.73	9.96, 11.57	9.57	8.82, 10.37	−1.17	0.03
Estradiol (pg/ml)	White	35.41	33.76, 37.14	28.94	27.34, 30.63	−6.47	<0.01
	Black	40.15	38.17, 42.22	38.94	34.88, 43.47	−1.21	0.61
	Mexican-American	33.41	31.03, 35.97	26.14	23.97, 28.51	−7.27	<0.01

[a] adjusted for age (1 year intervals), BMI, waist circumference, smoking, and alcohol use

[b] means for free testosterone, SHBG, 3α-diol-G, and estradiol are geometric means

[c] CI – confidence interval

Table 5

Difference in mean hormone concentrations by age and race/ethnicity, comparing NHANES 1988–1991 to 1999–2004.

		1988–1991		1999–2004			
	Age (years)	Mean[a, b]	95% CI[c]	Mean[a, b]	95% CI[c]	Difference	P-value
White							
Testosterone (ng/ml)	20–44	5.51	5.26, 5.76	5.20	4.90, 5.51	−0.31	0.06
	45–69	5.19	4.93, 5.45	5.35	5.04, 5.67	0.17	0.30
	≥ 70	5.21	4.70, 5.72	5.25	4.66, 5.84	0.04	0.85
Free testosterone (ng/ml)	20–44	0.102	0.096, 0.109	0.102	0.096, 0.108	0	0.93
	45–69	0.098	0.088, 0.110	0.095	0.087, 0.104	−0.003	0.61
	≥ 70	0.084	0.072, 0.097	0.070	0.057, 0.087	−0.014	0.09
SHBG (nmol/L)	20–44	36.07	33.56, 38.77	30.94	28.54, 33.53	−5.14	<0.01
	45–69	32.47	29.87, 35.28	35.29	31.99, 38.93	2.83	0.05
	≥ 70	36.12	30.40, 42.90	39.16	33.11, 46.32	3.04	0.13
3α-diol-G (ng/ml)	20–44	12.57	11.59, 13.64	9.68	8.88, 10.55	−2.89	<0.01
	45–69	11.57	10.26, 13.06	10.60	9.59, 11.71	−0.97	0.17
	≥ 70	12.32	10.09, 15.05	10.79	9.03, 12.89	−1.53	0.08
Estradiol (pg/ml)	20–44	35.14	33.11, 37.29	28.65	26.31, 31.19	−6.49	<0.01
	45–69	35.09	31.92, 38.58	29.37	26.96, 32.00	−5.72	<0.01
	≥ 70	38.46	34.02, 43.48	28.96	24.48, 34.27	−9.50	<0.01
Black							
Testosterone (ng/ml)	20–44	5.42	4.94, 5.91	5.97	5.55, 6.39	0.55	0.06
	45–69	5.37	4.82, 5.92	5.74	5.07, 6.42	0.38	0.37
	≥ 70	4.41	3.55, 5.28	6.26	4.14, 8.39	1.85	0.09
Free testosterone (ng/ml)	20–44	0.098	0.089, 0.107	0.112	0.101, 0.124	0.014	0.03
	45–69	0.102	0.090, 0.115	0.101	0.088, 0.115	−0.001	0.87
	≥ 70	0.069	0.048, 0.099	0.104	0.078, 0.137	0.035	0.05
SHBG (nmol/L)	20–44	35.21	32.02, 38.72	34.94	31.69, 38.52	−0.27	0.88
	45–69	33.08	29.71, 36.84	34.34	29.36, 40.17	1.26	0.64
	≥ 70	30.46	25.69, 36.11	29.98	23.81, 37.75	−0.48	0.88
3α-diol-G (ng/ml)	20–44	11.16	9.99, 12.46	10.16	9.24, 11.17	−1.00	0.15

		1988–1991		1999–2004			
	Age (years)	Mean[a, b]	95% CI[c]	Mean[a, b]	95% CI[c]	Difference	P-value
	45–69	10.54	8.19, 13.55	10.00	8.79, 11.37	−0.54	0.68
	≥ 70	9.77	6.91, 13.82	13.97	8.36, 23.36	4.20	0.19
Estradiol (pg/ml)	20–44	40.67	38.30, 43.18	38.97	34.31, 44.25	−1.70	0.54
	45–69	38.75	35.52, 42.26	38.03	31.58, 45.78	−0.72	0.84
	≥ 70	39.53	32.90, 47.49	44.41	31.62, 62.38	4.89	0.51
Mexican American							
Testosterone (ng/ml)	20–44	5.36	5.12, 5.60	5.07	4.65, 5.50	−0.28	0.16
	45–69	5.15	4.80, 5.49	5.16	4.44, 5.88	0.01	0.97
	≥ 70	5.52	4.51, 6.52	5.80	5.00, 6.60	0.28	0.62
Free testosterone (ng/ml)	20–44	0.104	0.099, 0.109	0.100	0.091, 0.109	−0.004	0.40
	45–69	0.094	0.086, 0.103	0.086	0.073, 0.101	−0.009	0.26
	≥ 70	0.104	0.086, 0.124	0.100	0.085, 0.117	−0.003	0.72
SHBG (nmol/L)	20–44	32.76	29.94, 35.85	32.54	29.20, 36.25	−0.23	0.90
	45–69	33.98	31.41, 36.77	34.94	29.32, 41.65	0.96	0.75
	≥ 70	32.33	26.43, 39.54	35.99	29.45, 43.98	3.66	0.35
3α-diol-G (ng/ml)	20–44	10.84	9.63, 12.21	9.57	8.54, 10.72	−1.27	0.09
	45–69	9.88	8.52, 11.45	9.24	8.05, 10.60	−0.64	0.48
	≥ 70	14.87	9.71, 22.77	12.07	10.17, 14.33	−2.79	0.33
Estradiol (pg/ml)	20–44	33.38	30.92, 36.03	25.88	23.47, 28.52	−7.50	<0.01
	45–69	32.31	28.69, 36.38	26.21	22.83, 30.10	−6.10	0.02
	≥ 70	37.93	31.13, 46.21	28.61	23.38, 35.02	−9.32	0.01

[a] adjusted for age (1 year intervals), BMI, waist circumference, smoking, and alcohol use

[b] means for free testosterone, SHBG, 3α-diol-G, and estradiol are geometric means

[c] CI – confidence interval