A Cross-Sectional Study of the Association of Age, Race and Ethnicity, and Body Mass Index with Sex Steroid Hormone Marker Profiles among Men in the National Health and Nutrition Examination Survey (NHANES III)

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Dear Editors:

The manuscript "A Cross-Sectional Study of the Association of Age, Race and Ethnicity, and Body Mass Index with Sex Steroid Hormone Marker Profiles among Men in the National Health and Nutrition Examination Survey (NHANES III)" may be interest of *The BMJ Open* readers. In summary, this study used a novel approach to investigate sex steroid hormones by building statistically determined hormone profiles using cluster analysis with nationally representative US data. The association with these hormone profiles by body mass index, race/ethnicity, and age groups were investigated in multinomial logistic regression models. Body mass index, race/ethnicity, and age groups were more strongly associated with different hormone profiles. Using hormone profiles instead of single hormone linear models is a more complete way to model chronic disease risk and future work is forthcoming.

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Sincerely,

Jamie Ritchey, MPH, PhD

TITLE

A Cross-Sectional Study of the Association of Age, Race and Ethnicity, and Body Mass Index with Sex Steroid Hormone Marker Profiles among Men in the National Health and Nutrition Examination Survey (NHANES III)

AUTHORS

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KEY WORDS cluster analysis, sex steroid hormones, race/ethnicity, Body mass index, NHANES

ABSTRACT

Objectives: Different levels of testosterone (T), 17-β estradiol (E), sex hormone binding globulin (SHBG) and androstanediol glucuronide (3-α diol G) hormone markers have been proposed to explain racial disparities in the rates of several chronic diseases. Epidemiologic studies of hormones stratified by race/ethnicity have been mixed. Since these markers are metabolically linked, assessing combined markers in multinomial logistic regression models may provide superior information on racial disparities compared to linear regression models.

Design: Cross-sectional survey

Setting: United States Third National Health and Nutrition Examination Survey (NHANES III)

Participants: 1,538 men, >17 years

Primary outcome measure: sex hormone profiles

Results: Cluster analysis was used to identify four statistically determined profiles with Blom-transformed T, E, SHBG, and 3-α diol G. We used these four profiles with multinomial logistic regression models to examine differences by race/ethnicity, age and BMI. Mexican American men >50 years were associated with the profile that had lowest T, E, and 3-α diol G levels compared to other profiles ($P<0.05$). Non-Hispanic Black, overweight (25-29.9 kg/m2), and obese (>30 kg/m2) men were most likely to be associated with the cluster with the lowest SHBG ($P<0.05$).

Conclusion: The associations of hormone profiles by race/ethnicity are novel, while findings by age and BMI groups are largely consistent with findings from single hormone studies. Future studies should examine hormone profiles in relation to chronic disease risk.

Trial registration: N/A

SUMMARY BOX

What is already known on this subject?

Past studies examining sex steroid hormones and their markers have been investigated in an effort to explain disparities in chronic disease rates by race/ethnicity. Epidemiologic studies using single hormones in linear models have been mixed, and it is still largely unclear if sex steroid hormones contribute to the racial disparities observed in chronic disease rates.

What does this study add?

Examining hormones in relation to race/ethnicity in singly in linear models as done in past studies does not account for the metabolic linkage between sex hormones and their markers. To account for metabolic linkage of sex hormones and markers, we created statistically determined hormone profiles by using cluster analysis. Then, we used these profiles as outcome variables in multinomial logistic regression models to determine if there were differences by age, BMI and race/ethnicity groups. Older men and Mexican American men were more likely to be associated with the profile that had lowest T, E, and 3-α diol G levels compared to other profiles ($P<0.05$), and Non-Hispanic Black, overweight (25-29.9 kg/m^2), and obese ($\geq$30 kg/m^2) men were most likely to be associated with the cluster with the lowest SHBG ($P<0.05$). Using statistically determined hormone marker profiles rather than hormones singly in models may better explain the relationship between sex steroid hormones and race/ethnicity in relation to chronic disease risk.

INTRODUCTION

Sex steroid hormones, testosterone (T) and 17-β estradiol (E), along with sex hormone binding globulin (SHBG), a carrier protein of T and E, and androstanediol glucuronide (3-α diol G) a metabolite used as a marker for T and dihydrotestosterone (DHT) metabolism, play critical roles in sexual development and body function [1-5]. These hormone markers are involved in muscle and bone growth, adipose tissue function and distribution [6-8]. Varying levels of these markers may contribute to chronic diseases, including, cardiovascular disease, osteoporosis, diabetes and cancers [5, 9-18]. It is well known that the incidence rates of these diseases differ by age and race/ethnicity [19]. Increasing BMI has been found to be associated with type II diabetes and cardiovascular diseases, although the impact of BMI on certain disease rates, like prostate cancer, is less clear [20-23]. Some differences in disease rates appear to be caused by age, race/ethnicity, and BMI, yet the underlying reasons for these associations remains unknown [19]. These underlying differences in disease rates by age, race/ethnicity, and BMI may be accounted for, at least in part, by differences in sex steroid hormone markers [19, 24-26].

Previous studies have investigated hormone marker levels singly by age, race/ethnicity, and BMI [5, 27-28]. Typically, with increasing age, T and E levels decline, and SHBG increases, although there is evidence to suggest that older men have hormone levels similar to younger men [29-36]. Higher hormone levels have been reported among Non-Hispanic Black men compared to Non-Hispanic Whites, although this finding is not consistent, and information for other racial/ethnic groups is scant [5, 8, 37-39]. With increasing BMI, T has been reported to decline and E and SHBG increase, yet these findings are not consistent across studies either [8, 27-28, 40-41]. Since E, T, SHBG, and 3-α diol G are known to be related through sex steroid

metabolism, investigating these markers combined may be more informative than investigating these markers singly. Therefore, we used cluster analysis to statistically determine which mean hormone marker levels cluster together to form specific profiles, and multinomial logistic regression was used to determine whether age, BMI, and race/ethnicity groups are more likely to be associated with different sex steroid hormone marker profiles.

MATERIALS AND METHODS

Study population

We utilized data from the National Health and Nutrition Examination Survey (NHANES) III conducted by the National Center for Health Statistics (NCHS), and these methods have been described previously [42-43]. Briefly, NHANES III is collected in two phases, and this study used the phase I data from 1988-1991 [42-43]. This cross-sectional survey was designed as a multistage stratified, clustered probability sample, the sampling frame includes U.S. residents ≥2 month of age, civilian, non-institutionalized population, and NHANES III over sampled those >65 years, Non-Hispanic Blacks and Mexican Americans [42].

The NHANES III study population was used to derive the analysis cohort. A total of 16,295 men were interviewed of which n=14,781 completed a mobile examination component (MEC) exam [42-43]. The NHANES III morning portion of the survey phase I (1988-1991), included n=2,417 men and n=1,637 provided samples [42-43]. We removed the males that were under 17 years of age and outliers for a final analysis cohort of n=1,528 men.

Exposure variables

Age, race/ethnicity and BMI are the exposures of interest. The NHANES III data obtained age and race/ethnicity information from the US Census survey 1990 to draw the sampling frame, so this information is 100% complete and was verified during the adult interview survey screening by NHANES III field staff [42-43]. BMI (weight in kg divided by height in m squared) was obtained from body measurements taken during the MEC [43]. BMI information is available for 99.5% (n=1,524) of men in the analysis cohort [42-43]. We constructed categories of BMI based on World Health Organization (WHO) guidelines: underweight <18.5, normal weight 18.5-24.9, overweight 25.0-29.9, and obese $\geq$30 kg/m^2 [44].

Outcome variable

Laboratory measurement methods in NHANES III have been described previously [42-43]. Briefly, NHANES III selected a random subset of n=1,637 men over 12 years of age during the 1988-1991 phase I survey collection, where morning blood samples were collected to measure serum levels of T, E, SHBG, and 3-α diol G using standard procedures [43]. As described previously, samples were centrifuged, serum was aliquotted and stored at -70 C [43]. Samples were randomly ordered and technicians were blinded to identity, age, and race/ethnicity [43]. The lowest detection limits by the electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer for the samples were: T 0.02 ng/ml, E 5.0 pg/mL, and SHBG 0.35 nmol/L [43]. Enzyme immunoassays were used for 3-α diol G and the lowest detection limits were 0.33 ng/mL [43]. The functional sensitivity, or the lowest analyte concentration that can be reproduced with a coefficient of variation >=20% for T is 0.12 ng/mL and 12 pg/mL for E [43]. Control samples were run at the start of the day, after every 100 samples, at the end of the day, once per reagent kit and after calibration [43]. Control samples fell within 2 Standard Deviations

(SD) at the start of the sample runs, but 3 SD are tolerated at prior control points [43]. Hormone marker data are included as continuous variables in the NHANES III dataset.

Blom-transformations of the laboratory results for T, E, SHBG, and 3-α diol G were used in this analysis. The Blom-transformed hormone marker variables were chosen for cluster analysis since these are rank approximations, and are unit-free, which makes the distribution of the four markers comparable. The Blom-transformed marker variables were moderately correlated ($r_{Spearman} < 0.50$) indicating that the unweighted observations are independent, and can be used for cluster analysis.

Model covariates

To adjust the regression models for lifestyle and dietary factors, we used data from the NHANES III adult, examination, and laboratory files for alcohol intake, smoking status, exercise amount, zinc, total calorie, total fat, total monosaturated fat, total polysaturated fat, total saturated fat, fiber, and lycopene intake. Data on lifestyle characteristics were collected from self-reported questionnaires and 24-hour dietary recalls [42-43]. Alcohol intake (grams) from 24-hour recalls was combined into three groups (Non-drinkers, drinkers, and missing). Smoking status is categorized into four levels, as men who do not smoke, men who smoke, but not every day, and men who are current everyday smokers of <35 cigarettes per day and ≥35 cigarettes per day. The exercise variable combined the total days per month a person participated in the following activities: walking/running, swimming, aerobic dance, other dance, calisthenics, gardening and yard work, weights, and other exercise activities. Because activities were all combined, it is possible to have multiple exercise events per day within one month. The count of exercise events for individuals was categorized into quartiles. Dietary factors, such as total fat,

monosaturated fat, polysaturated fat, saturated fat, fiber, total calories, and zinc intakes were taken from 24-hour dietary recalls. Serum lycopene concentration was measured in blood samples, and if levels were below detection (0.63 µg/ml) 0 was recorded. Lycopene concentrations and other food intake variables were grouped into quartiles.

The medical exam variables used in the models, included fasting status, exam day of the week, blood cholesterol level, aspartate aminotransferase, and alanine aminotransferase were from the MEC data. Fasting compliance was determined prior to blood and urine collection via questionnaire, and was not followed uniformly, for instance: <1% fasted for 20 hours or more, 91.8% fasted for 10.01-19.99 hours, 7.5% fasted for 10 hours or less, and <0.1% either did not fast or no value was available. Fasting time was not included in the final multinomial logistic model since additional analysis showed that fasting time did not change the odds ratios (ORs) of age, race/ethnicity, or BMI more than 10%. No minimum detection limits were presented for cholesterol, aspartate aminotransferase and alanine aminotransferase. Cholesterol, aspartate aminotransferase and alanine aminotransferase were categorized into quartiles for analysis.

Data analysis

All data analysis was conducted using SAS 9.2 (Cary, NC). K-means cluster analysis was chosen to create cluster profiles using Blom-transformed T, E, SHBG, and 3-α diol G over other exploratory methods since it assigns each observation only to one group, is based on least squares, tends to find clusters with roughly the same number of observations, and is robust to outliers in the data. The k-means procedure calculates statistics that can be used to determine the best number of k clusters, including: an approximate overall R-squared value, Pseudo F-Stat, and

Cubic Clustering Criteria (CCC). These statistics were employed to compare exploratory cluster solutions using 4 to 8 cluster groups on the unweighted data.

Multinomial logistic regression models using survey procedures were employed to examine how age, race/ethnicity, and BMI were associated with sex hormone profiles. Low SHBG served as the reference group since mean hormone values were most similar to the total population. Models were reduced by investigating the exposure variables (age, race/ethnicity, and BMI) for a 10% change in the ORs. Covariates included in the full models included age, race/ethnicity, BMI, exam day of the week, hours of fasting, aspartate aminotransferase, alanine aminotransferase, exercise level, smoking and drinking status, total calories, total fat, monosaturated fats, polyunsaturated fats, saturated fat, fiber, lycopene, and zinc intake.

RESULTS

We calculated the percentages (%) and 95% confidence intervals (CIs) for age, race/ethnicity, and BMI (n=1,528) (table 1). The majority of men in the cohort are 30-49 yrs. (42.25%), followed by 17-29 yrs. (29.05%), 50-69 yrs (21.18%), and over 70 yrs (7.52%). By race/ethnicity, men self-reported to be, non-Hispanic White (77.36%), and non-Hispanic Blacks (9.75%), Mexican American (5.25%), and all other races (7.64%). The highest proportions of men were either overweight, BMI 25.0-29.9, (39.73%) or normal weight, BMI 18.5-24.9 (38.45%), while 20.38% of men were considered obese, BMI $\geq$30.

We used cluster analysis to create hormone profiles from Blom-transformed T, E, SHBG, and 3-α diol G laboratory values, and only the four and five level cluster solutions performed well (data not shown). The pseudo F-statistic was improved over the five cluster solution, and the

CCC value was positive (1.2) for the four cluster solution (data not shown). The four cluster solution was used to create hormone profiles (table 2).

We examined the mean levels of Blom-transformed T, E, SHBG, and 3-α diol G for the hormone profiles and the total population to determine how the mean levels differed (table 2). The first cluster had lowest mean SHBG level of the groups, but the mean level of T, E, and 3-α diol G was the most similar to the total cohort (hereafter referred to as the 'low SHBG profile'). The second cluster had the highest mean 3-α diol G level compared to the other clusters (referred to as the 'high 3-α diol G profile'). The third cluster had the highest mean levels of T, E and SHBG (hereafter referred to as the 'high T, E, and SHBG profile'). The fourth cluster had lowest mean levels of T, E, and 3-α diol G compared to the other groups ('low T, E, and 3-α diol G profile').

We examined the percentages (%) and the 95% confidence intervals (95% CI) for the four hormone profiles by age, race/ethnicity, and BMI (table 3). The 'low SHBG profile' had a higher proportion of younger men, aged 17-29 yrs (37.0%) and 30-49 yrs (47.3%), and the 'low T, E, and 3-α diol G profile' had a statistically higher proportion of older men, 50-69 yrs (45.3%) and ≥70 yrs (22.8%). All clusters were predominately comprised of non-Hispanic Whites. The highest proportions of non-Hispanic Blacks were in the 'high T, E, and SHBG profile' (13.5%), and Mexican Americans in the 'low T, E, and 3-α diol G profile'. The 'low SHBG profile' had the highest proportion of obese men (38.1%).

Associations with hormone profiles and age, race/ethnicity, and BMI groups using multinomial logistic regression models were examined (table 4). The younger men (17-29 yrs) were associated with the 'low SHBG profile' (table 4). Men in the 'low T, E, and 3-α diol G

profile' were most associated with 50-69 yrs (OR =11.5, 95% confidence interval (CI): 4.74, 27.68) and 70 yrs or over (OR=24.3, 95% CI: 7.71, 76.82) (table 4). Non-Hispanic Black men had higher odds of being in the 'low SHBG profile' (OR=2.5, 95% CI: 1.30, 4.35), and Mexican American men were more strongly associated with the 'low T, E, and 3-α diol G profile' (OR=3.1, 95% CI: 1.69, 5.68). Obese men (BMI ≥30) were most likely to be associated with the 'low SHBG profile' compared to men with a normal BMI (18.5-24.9).

DISCUSSION

This is the first study to examine statistically determined sex steroid hormone marker profiles by age, BMI, and race/ethnicity groups. Applying our novel approach to studying sex steroid hormone levels among US men, we created four statistically determined clusters, described as: 'low SHBG', 'high 3-α diol G', 'high T, E, and SHBG', and 'low T, E, and 3-α diol G'. New evidence was found supporting associations with sex steroid hormone profiles for non-Hispanic Blacks and Mexican American men that differ from single hormone studies. Examining hormone profiles by age and BMI, our results largely agree with single hormone studies [5, 11, 21, 38, 45-47].

Men in our study associated with the 'low SHBG' profile were more likely to be younger (<17-29 yrs), obese (BMI ≥30), and non-Hispanic Black (table 4). Our findings indicate that the 'low SHBG profile' is more commonly associated with younger men (17-29 yrs) [5, 11, 38, 46], and lower SHBG levels were reported among obese men in single hormone studies [21, 45, 47]. By contrast, the discovery that non-Hispanic Blacks are more likely to be associated with a 'low SHBG profile' compared to non-Hispanic Whites and Mexicans is a novel. This result does not

agree with previous single hormone studies, which have reported no difference or higher levels of SHBG among non-Hispanic Blacks compared to non-Hispanic Whites [5, 11, 38, 46, 48].

The 'high 3-α diol G profile' associations with age and BMI are somewhat ambiguous compared to other profiles, while the 'high 3-α diol G profile' is more strongly associated with non-Hispanic Whites. Past studies investigating 3-α diol G have reported higher 3-α diol G activity among older men with a higher BMI [8, 10, 39, 49-56]. However, much stronger associations with older age and obesity are seen with other profiles compared to this profile [8, 10, 39, 49-56]. Among single hormone studies reported no difference in 3-α diol G levels by race among younger men [39], yet, older men have reported higher 3-α diol G activity in non-Hispanic Whites compared to non-Hispanic Blacks [5, 39, 51-53]. We found that Non-Hispanic Whites were more likely to be associated with 'higher 3-α diol G', which agrees with single hormone studies [5, 39, 51-53]. The reasons why hormone studies are largely consistent for higher 3-α diol G levels seen among older non-Hispanic White men, yet findings for other race/ethnicity groups are inconsistent is unclear [5, 11, 24, 38, 46, 48, 51-52, 57].

The men in the 'high T, E, and SHBG profile' are older than the first two profiles, are most likely to have a normal BMI, and there are not any differences between the race/ethnicity groups. Previous cross-sectional studies investigating T alone have reported high T levels among young men, yet other studies have indicated that high T levels are not found exclusively among young men [29-36, 58-60]. The results for the 'high T, E, and SHBG' profile are consistent with single hormone studies that reported higher T and SHBG among men with a normal BMI [61-62]. It has been hypothesized that higher sex steroid hormone levels are responsible for the racial disparities in rates of prostate cancer and other chronic diseases [24, 34, 38, 46, 63]. Despite a higher proportion of non-Hispanic Black men found in this profile (table 3), the lack of

association of the 'high T, E, and SHBG profile' with race/ethnicity (table 4) does not support this previously considered hypothesis [5, 24, 26, 34, 38, 46, 63].

The 'low T, E, and 3-α diol G profile' is more likely to be associated with men over 70 yrs and Mexican American men, while findings by BMI are less defined compared to other profiles. Previous studies have suggested that lowered T and E metabolism, and increasing SHBG levels are associated with older ages [29-36]. This is in agreement with our results, since 74% of men are over 50 yrs in this profile, and associations are strongest with older age groups (tables 3 and 4). Overweight and obesity have been associated with declines in T and SHBG, and despite the low T levels in this profile, the 'low SHBG profile' was more strongly associated with obesity [21, 61-62]. Past single hormone studies comparing T levels among Hispanics to non-Hispanics have conflicted, two reported no differences, one reported lower, and another reported higher levels [5, 24, 26, 51]. Although there were few studies comparing sex steroid hormones among Mexican Americans compared to non-Hispanic Whites studies conflict [5, 11, 24, 38, 46, 48, 51].

Differences in sex steroid hormone levels have been hypothesized to account for the differences in rates of chronic disease by age, BMI, and race/ethnicity. Lower SHBG levels have been associated with cardiovascular disease, type II diabetes, and metabolic syndrome, and since men in the 'low SHBG' profile tended to be obese, it is likely that they are at increased risk for chronic diseases, or already have one [5, 9, 18, 22, 61, 64]. Older age is also a risk factor for developing a number of chronic conditions, and the majority of older men (over 70 yrs) were in the 'low T, E, and 3-α diol G profile' [30, 36]. Yet, whether this profile denotes increased risk of disease or healthy aging is less clear. Past hypotheses have suggested higher hormone levels are responsible for the racial disparity in reported rates of prostate cancer and other chronic diseases.

However, our two profiles that had high hormone levels were not associated with non-Hispanic Blacks, indicating that higher hormone levels are not likely to be responsible for the racial disparities in reported rates [10, 24, 26, 39, 49-53, 55, 65-67]. Future investigations should validate these findings for sex hormone profiles by age, BMI, and race/ethnicity, and use sex steroid hormone profiles to assess chronic disease risk.

Our study has several strengths. NHANES III data is a nationally representative sample, so selection bias is minimized [42-43]. The NHANES III oversampled minorities and men over 65 years ensuring adequate numbers of men for analysis [42-43]. Our exposure variables were 99% complete [42-43]. Hormone levels were measured systematically using standard methods available at the time which did employ testing against control samples [42-43]. We were able to select only those men that provided samples in the morning to correct for daily hormone fluctuations, and control for day of the week the blood was drawn and fasting time [42-43].

The study also has several limitations. The dietary information is from self-report from 24-hour recall, and may not be an accurate consumption values [42-43]. Smoking status was also self-reported [42-43]. The study was only based on a single hormone measurement among men, which may not account for the daily complexity or serum hormone measurements over time. While the hormone profiles combine two major sex steroid hormones, a carrier protein and a metabolite, these profiles are still likely to be oversimplified.

In conclusion, specific sex steroid hormone marker profiles were more likely than others to be associated with one or more age, race/ethnicity, or BMI groups. Our findings for non-Hispanic Blacks and Mexican Americans are novel, since these groups have been hypothesized to be associated with higher sex steroid hormone levels, yet we found the opposite. Our findings by

age and BMI largely agreed with single hormone studies. Future work should investigate the relationship between sex steroid hormone markers and race/ethnicity, and how these profiles may influence racial disparities in chronic disease risk.

Table 1. Demographic information among men, US NHANES III 1988-1991

Demographic information	Total (n)	Percentage (%)
Age		
17-29	365	29.1
30-49	516	37.3
50-69	388	21.2
70 and over	259	7.5
Race/ethnicity		
Non-Hispanic White	689	77.4
Non-Hispanic Black	378	9.8
Mexican American	402	5.3
Other	59	7.6
Body Mass Index (kg/m^2)		
<18.5	21	1.4
18.5-24.9	555	38.5
25.0-29.9	623	39.7
≥30	328	20.4
Missing	1	<0.01

Abbreviations: US NHANES III, United States National Health and Nutrition Examination Survey III

Table 2. Blom-transformed sex steroid hormone marker mean levels in the total population and hormone marker profiles among American men, US NHANES III 1988-1991

Hormone Marker Group	Total (N)	Mean	SD	Absolute Difference[a]
Population Total				
Testosterone	1527	0.13	0.04	N/A
17-β Estradiol	1524	0.06	0.06	N/A
Sex Hormone Binding Globulin	1517	-0.15	0.05	N/A
Androstanediol Glucuronide	1505	0.16	0.04	N/A
Low SHBG profile				
Testosterone	415	-0.25	0.04	0.38
17-β Estradiol	417	0.32	0.06	0.26
Sex Hormone Binding Globulin	412	-1.10	0.06	1.25
Androstanediol Glucuronide	407	0.20	0.06	0.04
High 3-α diol G profile				
Testosterone	327	-0.02	0.04	0.11
17-β Estradiol	326	-0.67	0.07	0.61
Sex Hormone Binding Globulin	324	-0.08	0.05	0.23
Androstanediol Glucuronide	324	0.78	0.06	0.94
High T, E, and SHBG profile				
Testosterone	484	1.00	0.05	0.87
17-β Estradiol	480	0.68	0.05	0.62
Sex Hormone Binding Globulin	480	0.53	0.04	0.38
Androstanediol Glucuronide	476	0.15	0.05	0.01
Low T, E, and 3-α diol G				
Testosterone	298	-0.79	0.07	0.92
17-β Estradiol	298	-0.71	0.10	0.77
Sex Hormone Binding Globulin	298	0.25	0.07	0.40
Androstanediol Glucuronide	295	-0.98	0.04	1.14

Abbreviations: US NHANES III, United States National Health and Nutrition Examination Survey III; SD, Mean Standard deviation; N/A, not applicable

[a] Absolute difference between population total mean value and the individual hormone profile values

Table 3. Prevalence of age, race/ethnicity, and body mass index (kg/m^2) groups by hormone profiles among American men, US NHANES III 1988-1991

Demographic characteristics	'Low SHBG' %	'High 3-α diol G' %	'High T, E, SHBG' %	'Low T, E, 3-α diol G' %
Age (years)				
17-29	37.0	27.6	31.5	8.1
30-49	47.3	45.3	43.2	23.7
50-69	13.3	20.4	18.9	45.3
≥70	2.4	6.6	6.4	22.9
Race/ethnicity group				
Non-Hispanic White	76.3	83.1	73.5	74.6
Non-Hispanic Black	10.1	5.5	13.5	7.6
Mexican American	5.7	5.2	4.6	6.2
Other	2.9	3.2	2.4	3.6
Body mass index (kg/m^2)				
<18.5	0.5	1.8	2.3	0.8
18.5-24.9	23.3	35.6	55.6	37.9
25-29.9	38.1	45.7	35.6	42.7
≥30	38.1	16.9	6.5	18.6
Missing	0	0	0	<0.1

Abbreviations: US NHANES III, United States National Health and Nutrition Examination Survey III

Table 4. Hormone profile associations with age, race/ethnicity, and body mass index (kg/m^2) in reduced multinomial regression model[a], US NHANES III

Demographic characteristics	High 3-α diol G		High T, E, SHBG		Low T, E, 3-α diol G	
	OR	95% CI	OR	95% CI	OR	95% CI
Age (years)						
17-29	0.4 [b]	(0.2-0.7)	0.4 [b]	(0.3-0.6)	0.3 [b]	(0.1-1.0)
30-49[a]	1.0	-	1.0	-	1.0	-
50-69	1.9	(0.9-4.1)	2.3 [b]	(1.3-4.2)	11.5 [b]	(4.7-27.7)
70 and over	2.2 [b]	(1.0-4.7)	4.2 [b]	(1.9-8.9)	24.3 [b]	(7.7-76.8)
Race/ethnicity group						
non-Hispanic White[a]	1.0	-	1.0	-	1.0	-
non-Hispanic Black	0.4 [b]	(0.2-0.8)	1.0	(0.5-1.9)	0.7	(0.4-1.4)
Mexican American	1.5	(0.7-3.4)	1.4	(0.8-2.7)	3.1 [b]	(1.7-5.7)
Other	0.8	(0.3-2.2)	0.4 [b]	(0.2-0.8)	1.8	(0.7-4.4)
Body Mass Index (kg/m^2)						
<18.5	2.1	(0.8-5.3)	1.9	(0.2-24.6)	1.0	(0.1-13.6)
18.5-24.9[a]	1.0	N/A	1.0	N/A	1.0	-
25-29.9	0.6	(0.3-1.1)	0.3 [b]	(0.2-0.5)	0.4 [b]	(0.2-0.7)
≥30	0.2 [b]	(0.1-0.4)	0.05 [b]	(0.03-0.1)	0.1 [b]	(0.1-0.2)

Abbreviations: NHANES III, National Health and Nutrition Examination Survey III; 95% CI, 95% confidence interval; OR, odds ratio; BMI, Body mass index.

[a] Low SHBG profile is the reference group for multinomial logistic regression models

[b] Statistically significant, *P<0.05*

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CONFLICTS OF INTEREST

The authors have no competing interests.

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CONTRIBUTORSHIP STATEMENT

Each Author contributed to the study design, data analysis, and drafting of the final manuscript.

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A Cross-Sectional Study of the Association of Age, Race and Ethnicity, and Body Mass Index with Sex Steroid Hormone Marker Profiles among Men in the National Health and Nutrition Examination Survey (NHANES III)

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TITLE

A Cross-Sectional Study of the Association of Age, Race and Ethnicity, and Body Mass Index with Sex Steroid Hormone Marker Profiles among Men in the National Health and Nutrition Examination Survey (NHANES III)

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ABSTRACT

Objectives: Since sex hormone markers are metabolically linked, **examining sex steroid hormones singly may account for inconsistent findings by age, race/ethnicity, and body mass index across studies**. **First, these markers were statistically combined into profiles to account for the metabolic relationship between markers. Then, the relationships between sex steroid hormone profiles and age, race/ethnicity and body mass index were explored** in multinomial logistic regression models.

Design: Cross-sectional survey

Setting: United States Third National Health and Nutrition Examination Survey (NHANES III)

Participants: 1,538 men, >17 years

Primary outcome measure: sex hormone profiles

Results: Cluster analysis was used to identify four statistically determined profiles with Blom-transformed T, E, SHBG, and 3-α diol G. We used these four profiles with multinomial logistic regression models to examine differences by race/ethnicity, age and BMI. Mexican American men >50 years were associated with the profile that had lowest T, E, and 3-α diol G levels compared to other profiles (P<0.05). Non-Hispanic Black, overweight (25-29.9 kg/m2), and obese (>30 kg/m2) men were most likely to be associated with the cluster with the lowest SHBG (P<0.05).

Conclusion: The associations of **sex steroid** hormone profiles by race/ethnicity are novel, while the findings by age and BMI groups are largely consistent with **observations** from single

hormone studies. Future studies should **validate these hormone profile groups and investigate these profiles** in relation to **chronic diseases and certain cancers**.

Trial registration: N/A

SUMMARY BOX

What is already known on this subject?

Past studies examining sex steroid hormones and their markers have been investigated in an effort to explain disparities in chronic disease **and cancer** rates by race/ethnicity. Epidemiologic studies using single hormones in linear models have been mixed. **Presently,** it is still largely unclear if sex steroid hormones contribute to the racial disparities observed in chronic disease **and cancer** rates.

What does this study add?

Examining hormones in relation to race/ethnicity in singly in linear models as done in past studies does not account for the metabolic linkage between sex hormones and their markers. To account for metabolic linkage of sex hormones and markers, we created statistically determined hormone profiles by using cluster analysis. Then, we used these profiles as outcome variables in multinomial logistic regression models to determine if there were differences by age, BMI and race/ethnicity groups. Older men and Mexican American men were more likely to be associated with the profile that had lowest T, E, and 3-α diol G levels compared to other profiles ($P<0.05$), and Non-Hispanic Black, overweight (25-29.9 kg/m^2), and obese ($\geq$30 kg/m^2) men were most likely to be associated with the cluster with the lowest SHBG ($P<0.05$). **Findings by race/ethnicity groups and sex steroid hormones are novel compared to using linear models, while findings by age and BMI are largely consistent with single hormone studies.** Using statistically determined hormone marker profiles rather than hormones singly in models **may be a better approach to use to explain** the relationship between sex steroid hormones and race/ethnicity **and other potential risk factors for disease.**

INTRODUCTION

Sex steroid hormones, testosterone (T) and 17-β estradiol (E), along with sex hormone binding globulin (SHBG), a carrier protein of T and E, and androstanediol glucuronide (3-α diol G) a metabolite used as a marker for T and dihydrotestosterone (DHT) metabolism, play critical roles in sexual development and body function [1-5]. These hormone markers are involved in muscle and bone growth, adipose tissue function and distribution [6-8]. **Differences in the levels of sex hormone markers have been hypothesized to contribute to differences in several chronic diseases and prostate cancer rates observed by age, race/ethnicity, and BMI [9-20]. Yet, differences in sex steroid hormone marker levels by age, race/ethnicity, and BMI groups have yet to be fully clarified in the literature [21-35].**

Many previous studies have investigated **single sex** hormone marker levels **in linear regression models** by age, race/ethnicity, and BMI. Typically, with increasing age, T and E levels decline, and SHBG increases, although there is evidence to suggest that some older men have hormone marker levels similar to younger men [19, 21-22, 26, 28-29, 35-39]. **By race/ethnicity, higher hormone levels have been reported among non-Hispanic Black men compared to non-Hispanic Whites, although this finding is not consistent across studies; and, studies sex hormone markers among other racial/ethnic groups are scant** [5, 8, 30, 34-35, 40-42]. With increasing BMI, T has been reported to decline and E and SHBG increase, yet these findings are not consistent across studies [8, 21, 24, 26-28, 40]. **Based on somewhat inconsistent findings across these studies, it is possible that investigating factors that influence sex steroid hormone marker levels singly is inadequate.**

Sex hormone markers E, T, SHBG, and 3-α diol G are known to be related through sex steroid metabolism. **Since sex hormone markers are related, then differing hormone levels may be related to each other as well. Cluster analysis can identify underlying statistical patterns among sex hormone markers, which may be indicative of general patterns of sex steroid hormone markers among men. Investigating statistically related sex steroid hormone profiles** may **produce different associations** with **age, race/ethnicity, and BMI groups** than investigating these markers **singly in linear models**. Therefore, we used cluster analysis to statistically determine which mean hormone marker levels cluster together to form specific hormone profiles, and multinomial logistic regression to determine whether age, BMI, and race/ethnicity groups are more likely to be associated with different sex steroid hormone marker profiles.

MATERIALS AND METHODS

Study population

We utilized data from the National Health and Nutrition Examination Survey (NHANES) III conducted by the National Center for Health Statistics (NCHS), and these methods have been described previously [43-44]. Briefly, NHANES III is collected in two phases, and this study used the phase I data from 1988-1991. This cross-sectional survey was designed as a multistage stratified, clustered probability sample, the sampling frame includes U.S. residents ≥2 month of age, civilian, non-institutionalized population, and NHANES III over sampled those >65 years, Non-Hispanic Blacks and Mexican Americans.

The NHANES III study population was used to derive the analysis cohort. A total of 16,295 men were interviewed of which n=14,781 completed a mobile examination component (MEC)

exam [43-44]. The NHANES III morning portion of the survey phase I (1988-1991), included n=2,417 men and n=1,637 **that provided blood** samples. We removed the males that were under 17 years of age and **four outliers identified by box and whisker plot analysis** for a final analysis cohort of n=1,528 men.

Exposure variables

Age, race/ethnicity and BMI are the exposures of interest. The NHANES III data obtained age and race/ethnicity information from the US Census survey 1990 to draw the sampling frame, so this information is 100% complete and was verified during the adult interview survey screening by NHANES III field staff [43-44]. **Continuous age (in years) was categorized into the following groups: 17-29, 30-49, 50-69, and 70 and over. Race/ethnicity was categorized as White non-Hispanic, Black non-Hispanic, Mexican Americans and All others. Asian, American Indian/Alaskan Native, or Pacific Islanders are included in the other group. Whites and Blacks in the analysis reported non-Hispanic ethnicity. Mexican American is an ethnicity and may also report any race group (White, Black, Asian, American Indian/Alaskan Native, or Pacific Islander). Hispanics other than Mexican Americans were included in the other group, since there were few.** BMI (weight in kg divided by height in m squared) was obtained from body measurements taken during the MEC. BMI information is available for 99.5% (n=1,524) of men in the analysis cohort. We constructed categories of BMI based on World Health Organization (WHO) guidelines: underweight <18.5, normal weight 18.5-24.9, overweight 25.0-29.9, and obese ≥30 kg/m^2[45].

Outcome variable

Laboratory measurement methods in NHANES III have been described previously [43-44]. Briefly, NHANES III selected a random subset of n=1,637 men over 12 years of age during the 1988-1991 phase I survey collection, where morning blood samples were collected to measure serum levels of T, E, SHBG, and 3-α diol G using standard procedures. As described previously, samples were centrifuged, serum was aliquotted and stored at -70 C. Samples were randomly ordered and technicians were blinded to identity, age, and race/ethnicity. The lowest detection limits by the electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer for the samples were: T 0.02 ng/ml, E 5.0 pg/mL, and SHBG 0.35 nmol/L. Enzyme immunoassays were used for 3-α diol G and the lowest detection limits were 0.33 ng/mL. The functional sensitivity, or the lowest analyte concentration that can be reproduced with a coefficient of variation >=20% for T is 0.12 ng/mL and 12 pg/mL for E. Control samples were run at the start of the day, after every 100 samples, at the end of the day, once per reagent kit and after calibration. Control samples fell within 2 Standard Deviations (SD) at the start of the sample runs, but 3 SD are tolerated at prior control points. Hormone marker data are included as continuous variables in the NHANES III dataset.

Blom-transformations of the laboratory results for T, E, SHBG, and 3-α diol G were used in this analysis. Blom-transformed hormone marker variables were chosen for cluster analysis since these are rank approximations, and are unit-free, which makes the distribution of the four markers comparable [46]. The Blom-transformed marker variables were moderately correlated ($r_{Spearman}$ <0.50) indicating that the unweighted observations are independent, and can be used for cluster analysis.

Model covariates

To adjust the regression models for lifestyle and dietary factors, we used data from the NHANES III adult, examination, and laboratory files for alcohol intake, smoking status, exercise amount, zinc, total calorie, total fat, total monosaturated fat, total polysaturated fat, total saturated fat, fiber, were taken from 24-hour recall and lycopene intake was from blood samples [43-44]. Alcohol intake (grams) from 24-hour recalls was combined into three groups (Non-drinkers, drinkers, and missing). Smoking status is categorized into four levels, as men who do not smoke, men who smoke, but not every day, and men who are current everyday smokers of <35 cigarettes per day **or** ≥35 cigarettes per day. The exercise variable combined the total days per month a person participated in **exercise activities**. Serum lycopene concentration was measured in blood samples, and if levels were below detection (0.63 µg/ml) 0 was recorded. Exercise per month, lycopene concentrations, other food intake variables were grouped into quartiles.

The medical exam variables used in the models, included fasting status, exam day of the week, blood cholesterol level, aspartate aminotransferase, and alanine aminotransferase were from the MEC data. Fasting compliance was determined prior to blood and urine collection via questionnaire, and was not followed uniformly, for instance: <1% fasted for 20 hours or more, 91.8% fasted for 10.01-19.99 hours, 7.5% fasted for 10 hours or less, and <0.1% either did not fast or no value was available. No minimum detection limits were presented for cholesterol, aspartate aminotransferase and alanine aminotransferase. Cholesterol, aspartate aminotransferase and alanine aminotransferase were categorized into quartiles for analysis.

Data analysis

All data analysis was conducted using SAS 9.2 (Cary, NC). K-means cluster analysis was chosen to create cluster profiles using Blom-transformed T, E, SHBG, and 3-α diol G over other exploratory methods, since it assigns each observation only to one group, is based on least squares, tends to find clusters with roughly the same number of observations, and is robust to outliers in the data. The k-means procedure calculates statistics that can be used to determine the best number of k clusters, including: an approximate overall R-squared value, Pseudo F-Stat, and Cubic Clustering Criteria (CCC). These statistics were employed to compare exploratory cluster solutions using 4 to 8 cluster groups on the unweighted data**, since survey procedures are not available for cluster analysis in SAS 9.2**.

Multinomial logistic regression models using survey procedures (**accounting for weighted and stratified data**) were employed to examine how age, race/ethnicity, and BMI were associated with **the constructed** sex hormone profiles. Low SHBG served as the reference group since mean hormone values were most similar to the total population. Models were reduced by investigating the exposure variables (age, race/ethnicity, and BMI) for a 10% change in the ORs. Covariates included in the full models included age, race/ethnicity, BMI, exam day of the week, hours of fasting, aspartate aminotransferase, alanine aminotransferase, exercise level, smoking and drinking status, total calories, total fat, monosaturated fats, polyunsaturated fats, saturated fat, fiber, lycopene, and zinc intake.

RESULTS

We calculated the percentages (%) and 95% confidence intervals (CIs) for age, race/ethnicity, and BMI (n=1,528) (table 1). The majority of men in the cohort are 30-49 yrs. (42.25%),

followed by 17-29 yrs. (29.05%), 50-69 yrs (21.18%), and over 70 yrs (7.52%). By race/ethnicity, men self-reported to be, non-Hispanic White (77.36%), and non-Hispanic Blacks (9.75%), Mexican American (5.25%), and all other races (7.64%). The highest proportions of men were either overweight, BMI 25.0-29.9, (39.73%) or normal weight, BMI 18.5-24.9 (38.45%), while 20.38% of men were considered obese, BMI $\geq$30.

We used cluster analysis to create hormone profiles from Blom-transformed T, E, SHBG, and 3-α diol G laboratory values, and only the four and five level cluster solutions performed well (data not shown). The pseudo F-statistic was improved over the five cluster solution, and the CCC value was positive (1.2) for the four cluster solution (data not shown). The four cluster solution was used to create hormone profiles (table 2).

We examined the mean levels of Blom-transformed T, E, SHBG, and 3-α diol G for the hormone profiles and the total population to determine how the mean levels differed (table 2). The first cluster had lowest mean SHBG level of the groups, but the mean level of T, E, and 3-α diol G was the most similar to the total cohort (hereafter referred to as the 'low SHBG profile'). The second cluster had the highest mean 3-α diol G level compared to the other clusters (referred to as the 'high 3-α diol G profile'). The third cluster had the highest mean levels of T, E and SHBG (hereafter referred to as the 'high T, E, and SHBG profile'). The fourth cluster had lowest mean levels of T, E, and 3-α diol G compared to the other groups ('low T, E, and 3-α diol G profile').

Associations with hormone profiles and age, race/ethnicity, and BMI groups using **appropriately weighted** multinomial logistic regression models were examined (table 3). The younger men (17-29 yrs) were associated with the 'low SHBG profile'. Men in the 'low T, E,

and 3-α diol G profile' were most associated with 50-69 yrs (OR =11.5, 95% confidence interval (CI): 4.74, 27.68) and 70 yrs or over (OR=24.3, 95% CI: 7.71, 76.82). Non-Hispanic Black men had higher odds of being in the 'low SHBG profile' (OR=2.5, 95% CI: 1.30, 4.35), and Mexican American men were more strongly associated with the 'low T, E, and 3-α diol G profile' (OR=3.1, 95% CI: 1.69, 5.68). Obese men (BMI ≥30) were most likely to be associated with the 'low SHBG profile' compared to men with a normal BMI (18.5-24.9).

DISCUSSION

This is the first study to examine statistically determined sex steroid hormone marker profiles by age, BMI, and race/ethnicity groups. Applying our novel approach to studying sex steroid hormone levels among US men, we created four statistically determined clusters, described as: 'low SHBG', 'high 3-α diol G', 'high T, E, and SHBG', and 'low T, E, and 3-α diol G'. **Examining hormone profiles by age and BMI, our results largely agree with single hormone studies [5, 16, 21, 24, 30, 40, 47]. This study also found new evidence supporting differences in sex steroid hormone levels for non-Hispanic Blacks and Mexican American men using hormone profiles, and these observations differed from single hormone studies.**

Men in our study associated with the 'low SHBG' profile were more likely to be younger (<17-29 yrs), obese (BMI ≥30), and non-Hispanic Black (table 3). Our findings indicate that the 'low SHBG profile' is more commonly associated with younger men (17-29 yrs) [5, 16, 30, 40], and lower SHBG levels were reported among obese men in single hormone studies [21, 24, 47]. By contrast, the **observations** that non-Hispanic Blacks are more likely to be associated with a 'low SHBG profile' compared to non-Hispanic Whites and Mexicans **are new**. This result does not agree with previous single hormone studies, which have **dominantly** reported no differences

or higher levels of SHBG among non-Hispanic Blacks compared to non-Hispanic Whites [5, 16, 30-31, 40].

The 'high 3-α diol G profile' associations with age and BMI are somewhat ambiguous compared to other profiles, while the 'high 3-α diol G profile' is more strongly associated with non-Hispanic Whites. Past studies investigating 3-α diol G have reported higher 3-α diol G activity among older men with a higher BMI [8, 32-33, 41-42, 48-53]. However, much stronger associations with older age and obesity are seen with other profiles compared to this profile [8, 32-33, 41-42, 48-53]. Among **a** single hormone studies **that** reported no difference in 3-α diol G levels by race among younger men [41], **and in other studies** older men have reported higher 3-α diol G activity in non-Hispanic Whites compared to non-Hispanic Blacks [5, 41-42, 50-51]. We found that Non-Hispanic Whites were more likely to be associated with 'higher 3-α diol G', which agrees with single hormone studies [5, 41-42, 50-51]. The reasons why hormone studies are largely consistent for higher 3-α diol G levels seen among older non-Hispanic White men, while findings for other race/ethnicity groups are inconsistent is still unclear [5, 9, 16, 30-31, 34, 40, 42, 50].

The men in the 'high T, E, and SHBG profile' are older than the first two profiles, are most likely to have a normal BMI, and there are not any differences between the race/ethnicity groups. Previous cross-sectional studies investigating T alone have reported high T levels among young men, yet other studies have indicated that high T levels are not found exclusively among young men [19, 22, 26, 36-39, 54-57]. The results for the 'high T, E, and SHBG' profile are consistent with single hormone studies that reported higher T and SHBG among men with a normal BMI [58-59]. It has been hypothesized that higher sex steroid hormone levels are responsible for the racial disparities in rates of prostate cancer and other chronic diseases [9, 22, 30, 35, 40].

Despite a higher proportion of non-Hispanic Black men found in this profile (data not shown), the lack of association of the 'high T, E, and SHBG profile' with race/ethnicity (table 3) does not support this previously considered hypothesis [5, 9, 22, 30, 35, 40, 46].

The 'low T, E, and 3-α diol G profile' is more likely to be associated with men over 70 yrs and Mexican American men, while findings by BMI are less defined compared to other profiles. Previous studies have suggested that lowered T and E metabolism, and increasing SHBG levels are associated with older ages [19, 22, 26, 36-39, 54]. This is in agreement with our results, since 74% of men are over 50 yrs in this profile, and associations are strongest with older age groups (table 3). Overweight and obesity have been associated with declines in T and SHBG, and despite the low T levels in this profile, the 'low SHBG profile' was more strongly associated with obesity [24, 58-59]. Past single hormone studies comparing T levels among Hispanics to non-Hispanics have conflicted, two reported no differences, one reported lower, and another reported higher levels [5, 9, 42, 46]. Although there were few studies comparing sex steroid hormones among Mexican Americans compared to non-Hispanic Whites studies conflict [5, 9, 16, 30-31, 40, 42].

This study has several strengths. NHANES III data is a nationally representative sample, so selection bias is minimized [43-44]. The NHANES III oversampled minorities and men over 65 years ensuring adequate numbers of men for analysis [43-44]. Our exposure variables were 99% complete [43-44]. Hormone levels were measured systematically using standard methods available at the time which did employ testing against control samples [43-44]. We were able to select only those men that provided **blood** samples in the morning to correct for daily hormone fluctuations, and control for day of the week the blood was drawn and fasting time [43-44].

The study also has several limitations. The dietary information is from self-report from 24-hour recall, and may not be an accurate consumption values [43-44]. Smoking status was also self-reported [43-44]. The study was only based on a single hormone measurement among men, which may not account for the daily complexity or serum hormone measurements over time. While the hormone profiles combine two major sex steroid hormones, a carrier protein and a metabolite, these profiles are still likely to be an oversimplification **compared to hormone metabolism in the body**.

In conclusion, specific sex steroid hormone marker profiles were more likely than others to be associated with one or more age, race/ethnicity, or BMI groups. Our findings for non-Hispanic Blacks and Mexican Americans are novel, since these groups have often been suggested to be associated with higher sex steroid hormone levels, yet this study found the opposite. Our findings by age and BMI largely agreed with most single hormone studies. **Future work should investigate the relationship between sex steroid hormone markers and chronic disease risk.**

Table 1. Demographic information among men, US NHANES III 1988-1991

Demographic information	Total (n)	Percentage (%)
Age		
17-29	365	29.1
30-49	516	37.3
50-69	388	21.2
70 and over	259	7.5
Race/ethnicity		
Non-Hispanic White	689	77.4
Non-Hispanic Black	378	9.8
Mexican American	402	5.3
Other	59	7.6
Body Mass Index (kg/m^2)		
<18.5	21	1.4
18.5-24.9	555	38.5
25.0-29.9	623	39.7
≥30	328	20.4
Missing	1	<0.01

Abbreviations: US NHANES III, United States National Health and Nutrition Examination Survey III

Table 2. Blom-transformed sex steroid hormone marker mean levels in the total population and hormone marker profiles among American men, US NHANES III 1988-1991

Hormone Marker Group	Total (N)	Mean	SD
Population Total			
Testosterone	1527	0.13	0.04
17-β Estradiol	1524	0.06	0.06
Sex Hormone Binding Globulin	1517	-0.15	0.05
Androstanediol Glucuronide	1505	0.16	0.04
Low SHBG profile			
Testosterone	415	-0.25	0.04
17-β Estradiol	417	0.32	0.06
Sex Hormone Binding Globulin	412	-1.10	0.06
Androstanediol Glucuronide	407	0.20	0.06
High 3-α diol G profile			
Testosterone	327	-0.02	0.04
17-β Estradiol	326	-0.67	0.07
Sex Hormone Binding Globulin	324	-0.08	0.05
Androstanediol Glucuronide	324	0.78	0.06
High T, E, and SHBG profile			
Testosterone	484	1.00	0.05
17-β Estradiol	480	0.68	0.05
Sex Hormone Binding Globulin	480	0.53	0.04
Androstanediol Glucuronide	476	0.15	0.05
Low T, E, and 3-α diol G			
Testosterone	298	-0.79	0.07
17-β Estradiol	298	-0.71	0.10
Sex Hormone Binding Globulin	298	0.25	0.07
Androstanediol Glucuronide	295	-0.98	0.04

Abbreviations: US NHANES III, United States National Health and Nutrition Examination Survey III; SD, Mean Standard deviation; N/A, not applicable

[a] Absolute difference between population total mean value and the individual hormone profile values

Table 3. Hormone profile associations with age, race/ethnicity, and body mass index (kg/m^2) in reduced multinomial regression model[a, b, c], US NHANES III

Demographic characteristics	High 3-α diol G		High T, E, SHBG		Low T, E, 3-α diol G	
	OR	95% CI	OR	95% CI	OR	95% CI
Age (years)						
17-29	0.4 [d]	(0.2-0.7)	0.4 [d]	(0.3-0.6)	0.3 [d]	(0.1-1.0)
30-49[a]	1.0	-	1.0	-	1.0	-
50-69	1.9	(0.9-4.1)	2.3 [d]	(1.3-4.2)	11.5 [d]	(4.7-27.7)
70 and over	2.2 [d]	(1.0-4.7)	4.2 [d]	(1.9-8.9)	24.3 [d]	(7.7-76.8)
Race/ethnicity group						
non-Hispanic White[a]	1.0	-	1.0	-	1.0	-
non-Hispanic Black	0.4 [d]	(0.2-0.8)	1.0	(0.5-1.9)	0.7	(0.4-1.4)
Mexican American	1.5	(0.7-3.4)	1.4	(0.8-2.7)	3.1 [d]	(1.7-5.7)
Other	0.8	(0.3-2.2)	0.4 [d]	(0.2-0.8)	1.8	(0.7-4.4)
Body Mass Index (kg/m^2)						
<18.5	2.1	(0.8-5.3)	1.9	(0.2-24.6)	1.0	(0.1-13.6)
18.5-24.9[a]	1.0	N/A	1.0	N/A	1.0	-
25-29.9	0.6	(0.3-1.1)	0.3 [d]	(0.2-0.5)	0.4 [d]	(0.2-0.7)
≥30	0.2 [d]	(0.1-0.4)	0.05 [d]	(0.03-0.1)	0.1 [d]	(0.1-0.2)

Abbreviations: NHANES III, National Health and Nutrition Examination Survey III; 95% CI, 95% confidence interval; OR, odds ratio; BMI, Body mass index.

[a] Low SHBG profile is the reference group for multinomial logistic regression models

[b] The reduced model is adjusted for: age, race/ethnicity, BMI, exam day of the week, exercise level, smoking and drinking status, total calories, total fat, monosaturated fats, polyunsaturated fats, saturated fat, fiber, lycopene, and zinc intake

[c] Models used appropriate strata and weighting for national representation

[d] Statistically significant, *P<0.05*

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Each Author contributed to the study design, data analysis, and drafting of the final manuscript.

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A Cross-Sectional Study of the Association of Age, Race and Ethnicity, and Body Mass Index with Sex Steroid Hormone Marker Profiles among Men in the National Health and Nutrition Examination Survey (NHANES III)

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TITLE

A Cross-Sectional Study of the Association of Age, Race and Ethnicity, and Body Mass Index with Sex Steroid Hormone Marker Profiles among Men in the National Health and Nutrition Examination Survey (NHANES III)

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ABSTRACT

Objectives: Since sex hormone markers are metabolically linked, examining sex steroid hormones singly may account for inconsistent findings by age, race/ethnicity, and body mass index across studies. First, these markers were statistically combined into profiles to account for the metabolic relationship between markers. Then, the relationships between sex steroid hormone profiles and age, race/ethnicity and body mass index were explored in multinomial logistic regression models.

Design: Cross-sectional survey

Setting: United States **(U.S.)** Third National Health and Nutrition Examination Survey (NHANES III)

Participants: 1,538 men, >17 years

Primary outcome measure: sex hormone profiles

Results: Cluster analysis was used to identify four statistically determined profiles with Blom-transformed T, E, SHBG, and 3-α diol G. We used these four profiles with multinomial logistic regression models to examine differences by race/ethnicity, age and BMI. Mexican American men >50 years were associated with the profile that had lowest T, E, and 3-α diol G levels compared to other profiles ($P<0.05$). Non-Hispanic Black, overweight (25-29.9 kg/m2), and obese (>30 kg/m2) men were most likely to be associated with the cluster with the lowest SHBG ($p<0.05$).

Conclusion: The associations of sex steroid hormone profiles by race/ethnicity are novel, while the findings by age and BMI groups are largely consistent with observations from single

hormone studies. Future studies should validate these hormone profile groups and investigate these profiles in relation to chronic diseases and certain cancers.

Trial registration: N/A

Article Summary

Article focus

1. Using cluster analysis, can unique groups of sex steroid hormones be formed among a nationally representative sample of men which would take into account that these hormone marker levels are related?
2. In multinomial logistic regression models rather than linear models, are age, race/ethnicity, and body mass index groups more strongly associated with different statistically determined sex steroid hormone clusters?
3. How do the associations from multinomial logistic regression models compare to findings between sex steroid hormones and age, race/ethnicity, and BMI groups using linear regression models?

Key messages

1. To take into account the fact that sex steroid hormone marker levels are related, four distinct sex steroid hormone profiles were statistically determined using cluster analysis, described as: 'low SHBG', 'high 3-α diol G', 'high T, E, and SHBG', and 'low T, E, and 3-α diol G' profiles.
2. Mexican American men >50 years were associated with the profile that had lowest T, E, and 3-α diol G levels compared to other profiles ($P<0.05$). Non-Hispanic Black, overweight (25-29.9 kg/m2), and obese (>30 kg/m2) men were most likely to be associated with the cluster with the lowest SHBG ($P<0.05$).
3. The associations of sex steroid hormone profiles by race/ethnicity are novel, while findings by age and BMI groups are largely consistent with results from single hormone studies. Future studies should examine hormone profiles in relation to chronic disease risk.

Strengths and Limitations

1. Nationally representative sample of men in the US where minority groups were oversampled to ensure adequate representation in study analyses.
2. Hormone marker values were used from a single measurement and covariates like diet and smoking were taken from self-reported data.

INTRODUCTION

Sex steroid hormones, testosterone (T) and 17-β estradiol (E), along with sex hormone binding globulin (SHBG), a carrier protein of T and E, and androstanediol glucuronide (3-α diol G) a metabolite used as a marker for T and dihydrotestosterone (DHT) metabolism, play critical roles in sexual development and body function [1-5]. These hormone markers are involved in muscle and bone growth, adipose tissue function and distribution [6-8]. Differences in the levels of sex hormone markers have been hypothesized to contribute to differences in several chronic diseases and prostate cancer rates observed by age, race/ethnicity, and BMI [9-20]. Yet, differences in sex steroid hormone marker levels by age, race/ethnicity, and BMI groups have yet to be fully clarified in the literature [21-35].

Many previous studies have investigated single sex hormone marker levels in linear regression models by age, race/ethnicity, and BMI. Typically, with increasing age, T and E levels decline, and SHBG increases, although there was evidence to suggest that some older men have hormone marker levels similar to younger men [19, 21-22, 26, 28-29, 35-39]. By race/ethnicity, higher hormone levels have been reported among non-Hispanic Black men compared to non-Hispanic Whites, although this finding was not consistent across studies; and, studies sex hormone markers among other racial/ethnic groups were scant [5, 8, 30, 34-35, 40-42]. With increasing BMI, T has been reported to decline and E and SHBG increase, yet these findings are not consistent across studies [8, 21, 24, 26-28, 40]. Based on somewhat inconsistent findings across these studies, it is possible that investigating factors that influence sex steroid hormone marker levels singly was inadequate.

Sex hormone markers E, T, SHBG, and 3-α diol G are known to be related through sex steroid metabolism. Since sex hormone markers are related, then differing hormone levels may be related to each other as well. Cluster analysis can identify underlying statistical patterns among sex hormone markers, which may be indicative of general patterns of sex steroid hormone markers among men. Investigating statistically related sex steroid hormone profiles may produce different associations with age, race/ethnicity, and BMI groups than investigating these markers singly in linear models. Therefore, we used cluster analysis to statistically determine which mean hormone marker levels cluster together to form specific hormone profiles, and multinomial logistic regression to determine whether age, BMI, and race/ethnicity groups were more likely to be associated with different sex steroid hormone marker profiles.

MATERIALS AND METHODS

Study population

We utilized data from the National Health and Nutrition Examination Survey (NHANES) III conducted by the National Center for Health Statistics (NCHS), and these methods have been described previously [43-44]. Briefly, NHANES III was collected in two phases, and this study used the phase I data from 1988-1991. This cross-sectional survey was designed as a multistage stratified, clustered probability sample, the sampling frame includes U.S. residents ≥2 months of age, civilian, non-institutionalized population, and NHANES III over sampled those >65 years, Non-Hispanic Blacks and Mexican Americans.

The NHANES III study population was used to derive the analysis cohort. A total of 16,295 men were interviewed of which n=14,781 completed a mobile examination component (MEC) exam [43-44]. The NHANES III morning portion of the survey phase I (1988-1991), included

n=2,417 men and n=1,637 that provided blood samples. We removed the males that were under 17 years of age and four outliers with high 17-β estradiol levels identified by box and whisker plot analysis for a final analysis cohort of n=1,528 men.

Exposure variables

Age, race/ethnicity and BMI were the exposures of interest. The NHANES III data obtained age and race/ethnicity information from the U.S**.** Census survey 1990 to draw the sampling frame, so this information was 100% complete and was verified during the adult interview survey screening by NHANES III field staff [43-44]. Continuous age (in years) was categorized into the following groups: 17-29, 30-49, 50-69, and 70 and over. Race/ethnicity was categorized as White non-Hispanic, Black non-Hispanic, Mexican Americans and All others. Asian, American Indian/Alaskan Native, or Pacific Islanders were included in the other group. Whites and Blacks in the analysis reported non-Hispanic ethnicity. Mexican American is considered an ethnicity, and may also report any race group (White, Black, Asian, American Indian/Alaskan Native, or Pacific Islander). Hispanics other than Mexican Americans were included in the other group, since there were few**.** BMI (weight in kg divided by height in m squared) was obtained from body measurements taken during the MEC. BMI information is available for 99.5% (n=1,524) of men in the analysis cohort. Categories of BMI were constructed based on World Health Organization (WHO) guidelines: underweight <18.5, normal weight 18.5-24.9, overweight 25.0-29.9, and obese ≥30 kg/m^2 [45].

Outcome variable

Laboratory measurement methods in NHANES III have been described previously [43-44]. Briefly, NHANES III selected a random subset of n=1,637 men over 12 years of age during the 1988-1991 phase I survey collection, where morning blood samples were collected to measure serum levels of T, E, SHBG, and 3-α diol G using standard procedures. As described previously, samples were centrifuged, serum was aliquotted and stored at -70 C°. Samples were randomly ordered and technicians were blinded to identity, age, and race/ethnicity. The lowest detection limits by the electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer for the samples were: T 0.02 ng/ml, E 5.0 pg/mL, and SHBG 0.35 nmol/L. Enzyme immunoassays were used for 3-α diol G and the lowest detection limits were 0.33 ng/mL. The functional sensitivity, or the lowest analyte concentration that can be reproduced with a coefficient of variation ≥20% for T **was** 0.12 ng/mL and 12 pg/mL for E. Control samples were run at the start of the day, after every 100 samples, at the end of the day, once per reagent kit and after calibration. Control samples fell within 2 standard deviations (SD) at the start of the sample runs, but 3 SD were tolerated at prior control points. Hormone marker data were included as continuous variables in the NHANES III dataset.

Blom-transformations of the laboratory results for T, E, SHBG, and 3-α diol G were used in this analysis. Blom-transformed hormone marker variables were chosen for cluster analysis since these are rank approximations, and were unit-free, which makes the distribution of the four markers comparable [46]. The Blom-transformed marker variables were moderately correlated ($r_{Spearman} < 0.50$) indicating that the unweighted observations are independent, and can be used for cluster analysis.

Model covariates

To adjust the regression models for lifestyle and dietary factors, we used data from the NHANES III adult, examination, and laboratory files. Alcohol intake, smoking status, exercise amount, zinc, total calorie, total fat, total monosaturated fat, total polysaturated fat, total saturated fat, fiber, were taken from 24-hour recall surveys, which captured food intake from the past twenty four hours [43-44]. Lycopene intake was from blood samples since it was not available from 24-hour recall surveys**.** Alcohol intake (grams) was combined into three groups (**n**on-drinkers, drinkers, and missing). Smoking status was categorized into four levels, as men who do not smoke, men who smoke, but not every day, and men who are current everyday smokers of <35 cigarettes per day **or** ≥35 cigarettes per day. The exercise variable combined the total days per month a person participated in exercise activities. Serum lycopene concentration was measured in blood samples, and if levels were below detection (0.63 μg/ml) 0 was recorded. Exercise per month, lycopene concentrations, other food intake variables were grouped into quartiles.

The medical exam variables used in the models, included fasting status, exam day of the week, blood cholesterol level, aspartate aminotransferase, and alanine aminotransferase were from the MEC data. Fasting compliance was determined prior to blood and urine collection via questionnaire, and was not followed uniformly, for instance: <1% fasted for 20 hours or more, 91.8% fasted for 10.01-19.99 hours, 7.5% fasted for 10 hours or less, and <0.1% either did not fast or no value was available. No minimum detection limits were presented for cholesterol, aspartate aminotransferase and alanine aminotransferase. Cholesterol, aspartate aminotransferase and alanine aminotransferase were categorized into quartiles for analysis.

Data analysis

All data analysis was conducted using SAS 9.2 (Cary, NC). K-means cluster analysis was chosen to create cluster profiles using Blom-transformed T, E, SHBG, and 3-α diol G over other exploratory methods, since it assigns each observation only to one group, **is** based on least squares, tends to find clusters with roughly the same number of observations, and is robust to outliers in the data. The k-means procedure calculates statistics that can be used to determine the best number of k clusters, including: an approximate overall R-squared value, Pseudo F-Stat, and Cubic Clustering Criteria (CCC). These statistics were employed to compare exploratory cluster solutions using 4 to 8 cluster groups on the unweighted data**,** since survey procedures were not available for cluster analysis in SAS 9.2.

Multinomial logistic regression models using survey procedures **(**accounting for weighted and stratified data**)** were employed to examine how age, race/ethnicity, and BMI were associated with the constructed sex hormone profiles. Low SHBG served as the reference group since mean hormone values were most similar to the total population. Models were reduced by investigating the exposure variables (age, race/ethnicity, and BMI) for a 10% change in the odds ratios (ORs). Covariates included in the full models included age, race/ethnicity, BMI, exam day of the week, hours of fasting, aspartate aminotransferase, alanine aminotransferase, cholesterol levels, exercise level, smoking and drinking status, total calories, total fat, monosaturated fats, polyunsaturated fats, saturated fat, fiber, lycopene, and zinc intake.

RESULTS

We calculated the percentages (%) and 95% confidence intervals (CIs) for age, race/ethnicity, and BMI (n=1,528) (table 1). The majority of men in the cohort were 30-49 yrs. (42.25%),

followed by 17-29 yrs. (29.05%), 50-69 yrs (21.18%), and over 70 yrs (7.52%). By race/ethnicity, men self-reported to be, non-Hispanic White (77.36%), and non-Hispanic Blacks (9.75%), Mexican American (5.25%), and all other races (7.64%). The highest proportions of men were either overweight, BMI 25.0-29.9, (39.73%) or normal weight, BMI 18.5-24.9 (38.45%), while 20.38% of men were considered obese, BMI $\geq$30.

We used cluster analysis to create hormone profiles from Blom-transformed T, E, SHBG, and 3-α diol G laboratory values, and only the four and five level cluster solutions performed well (data not shown). The pseudo F-statistic was improved over the five cluster solution, and the CCC value was positive (1.2) for the four cluster solution (data not shown). The four cluster solution was used to create hormone profiles (table 2).

We examined the mean levels of Blom-transformed T, E, SHBG, and 3-α diol G for the hormone profiles and the total population to determine how the mean levels differed (table 2). The first cluster had lowest mean SHBG level of the groups, but the mean level of T, E, and 3-α diol G was the most similar to the total cohort (hereafter referred to as the 'low SHBG profile'). The second cluster had the highest mean 3-α diol G level compared to the other clusters (referred to as the 'high 3-α diol G profile'). The third cluster had the highest mean levels of T, E and SHBG (hereafter referred to as the 'high T, E, and SHBG profile'). The fourth cluster had lowest mean levels of T, E, and 3-α diol G compared to the other groups ('low T, E, and 3-α diol G profile').

Associations with hormone profiles and age, race/ethnicity, and BMI groups using weighted multinomial logistic regression models were examined (table 3). The younger men (17-29 yrs) were associated with the 'low SHBG profile'. Men in the 'low T, E, and 3-α diol G profile' were

most associated with 50-69 yrs (OR =11.5, 95% confidence interval (CI): 4.74, 27.68) and 70 yrs or over (OR=24.3, 95% CI: 7.71, 76.82). Non-Hispanic Black men had higher odds of being in the 'low SHBG profile' (OR=2.5, 95% CI: 1.30, 4.35), and Mexican American men were more strongly associated with the 'low T, E, and 3-α diol G profile' (OR=3.1, 95% CI: 1.69, 5.68). Obese men (BMI ≥30) were most likely to be associated with the referent 'low SHBG profile' compared to men with a normal BMI (18.5-24.9) in all other profiles.

DISCUSSION

This is the first study to examine statistically determined sex steroid hormone marker profiles by age, BMI, and race/ethnicity groups. Applying our novel approach to studying sex steroid hormone levels among U.S. men, we created four statistically determined clusters, described as: 'low SHBG', 'high 3-α diol G', 'high T, E, and SHBG', and 'low T, E, and 3-α diol G'. Examining hormone profiles by age and BMI, our results largely agree with single hormone studies [5, 16, 21, 24, 30, 40, 47]. This study also found new evidence supporting differences in sex steroid hormone levels for non-Hispanic Blacks and Mexican American men using hormone profiles, and these observations differed from single hormone studies.

Men in our study associated with the 'low SHBG' profile were more likely to be younger (<17-29 yrs), obese (BMI ≥30), and non-Hispanic Black (table 3). Our findings indicate that the 'low SHBG profile' was more commonly associated with younger men (17-29 yrs) [5, 16, 30, 40], and lower SHBG levels were reported among obese men in single hormone studies [21, 24, 47]. By contrast, the observations that non-Hispanic Blacks were more likely to be associated with a 'low SHBG profile' compared to non-Hispanic Whites and Mexicans were new. This result does not agree with previous single hormone studies, which have dominantly reported no

differences or higher levels of SHBG among non-Hispanic Blacks compared to non-Hispanic Whites [5, 16, 30-31, 40].

The 'high 3-α diol G profile' associations with age and BMI were somewhat ambiguous compared to other profiles, while the 'high 3-α diol G profile' was more strongly associated with non-Hispanic Whites. Past studies investigating 3-α diol G have reported higher 3-α diol G activity among older men with a higher BMI [8, 32-33, 41-42, 48-53]. However, much stronger associations with older age and obesity were seen with other profiles compared to this profile [8, 32-33, 41-42, 48-53]. Some single hormone studies reported no difference in 3-α diol G levels by race among younger men [41], yet in other studies older men have reported higher 3-α diol G activity in non-Hispanic Whites compared to non-Hispanic Blacks which agrees with the findings in this study [5, 41-42, 50-51]. The reasons why hormone studies were largely consistent for higher 3-α diol G levels seen among older non-Hispanic White men, while findings for other race/ethnicity groups were inconsistent was still unclear [5, 9, 16, 30-31, 34, 40, 42, 50].

The men in the 'high T, E, and SHBG profile' were older than the first two profiles, were most likely to have a normal BMI, and there were not any differences between the race/ethnicity groups. Previous cross-sectional studies investigating T alone have reported high T levels among young men, yet other studies have indicated that high T levels were not found exclusively among young men [19, 22, 26, 36-39, 54-57]. The results for the 'high T, E, and SHBG' profile were consistent with single hormone studies that reported higher T and SHBG among men with a normal BMI [58-59]. Past studies have hypothesized that higher sex steroid hormones (T and E) were responsible for the racial disparities observed in the rates of prostate cancer [9, 22, 30, 35, 40]. Despite a higher proportion of non-Hispanic Black men found in the 'high T, E, and SHBG

profile', non-Hispanic Black men were not associated with this profile (data not shown). These findings do not support this previously considered hypothesis that sex steroid hormone levels are higher among non-Hispanic Black men compared to other race/ethnicity groups [5, 9, 22, 30, 35, 40, 46].

The 'low T, E, and 3-α diol G profile' was more likely to be associated with men over 70 yrs and Mexican American men, while findings by BMI were less defined compared to other profiles. Previous studies have suggested that lowered T and E metabolism, and increasing SHBG levels were associated with older ages [19, 22, 26, 36-39, 54]. This was in agreement with our results, since 74% of men are over 50 yrs in this profile, and associations were strongest with older age groups (table 3). Overweight and obesity have been associated with declines in T and SHBG, and despite the low T levels in this profile, the 'low SHBG profile' was more strongly associated with obesity [24, 58-59]. Past single hormone studies specifically comparing T levels among Hispanics to non-Hispanics have conflicted, two reported no differences, one reported lower, and another reported higher levels [5, 9, 42, 46]. Although there were few studies comparing sex steroid hormones among Mexican Americans compared to non-Hispanic Whites these findings from these studies conflict [5, 9, 16, 30-31, 40, 42].

This study has several strengths. NHANES III data was a nationally representative sample, so selection bias was minimized [43-44]. The NHANES III oversampled minorities and men over 65 years ensuring adequate numbers of men for analysis [43-44]. Our exposure variables were 99% complete [43-44]. Hormone levels were measured systematically using standard methods available at the time which did employ testing against control samples [43-44]. We were able to select only those men that provided blood samples in the morning to correct for

daily hormone fluctuations, and control for day of the week the blood was drawn and fasting time [43-44].

The study also has several limitations. The dietary information was from self-reported 24-hour recall surveys, and may not reflect a man's true dietary behaviors [43-44]. Smoking status was also self-reported [43-44]. The study was only based on a single hormone measurement among men, which may not account for the daily complexity or serum hormone measurements over time. While the hormone profiles combine two major sex steroid hormones, a carrier protein and a metabolite, these profiles are still likely to be an oversimplification compared to hormone metabolism in the body.

In conclusion, specific sex steroid hormone marker profiles were more likely than others to be associated with one or more age, race/ethnicity, or BMI groups. Our findings for non-Hispanic Blacks and Mexican Americans are novel, since these groups have often been suggested to be associated with higher sex steroid hormone levels, yet this study found the opposite. Our findings by age and BMI largely agreed with most single hormone studies. The observed race/ethnicity differences across the hormone profiles in the current analysis, suggest that when accounting for the relationship between sex steroid hormone markers, race/ethnicity differences become apparent. Further research is necessary to determine if sex steroid hormone profiles contribute to the increased risk of several cancers and chronic diseases observed by race/ethnicity.

Table 1. Demographic information among men, **U.S.** NHANES III 1988-1991

Demographic information	Total (n)	Percentage (%)
Age		
17-29	365	29.1
30-49	516	37.3
50-69	388	21.2
70 and over	259	7.5
Race/ethnicity		
Non-Hispanic White	689	77.4
Non-Hispanic Black	378	9.8
Mexican American	402	5.3
Other	59	7.6
Body Mass Index (kg/m^2)		
<18.5	21	1.4
18.5-24.9	555	38.5
25.0-29.9	623	39.7
≥30	328	20.4
Missing	1	<0.01

Abbreviations: **U.S.** NHANES III, United States National Health and Nutrition Examination Survey III

Table 2. Blom-transformed sex steroid hormone marker mean levels in the total population and hormone marker profiles among American men, **U.S.** NHANES III 1988-1991

Hormone Marker Group	Total (N)	Mean	SD
Population Total			
Testosterone	1527	0.13	0.04
17-β Estradiol	1524	0.06	0.06
Sex Hormone Binding Globulin	1517	-0.15	0.05
Androstanediol Glucuronide	1505	0.16	0.04
Low SHBG profile			
Testosterone	415	-0.25	0.04
17-β Estradiol	417	0.32	0.06
Sex Hormone Binding Globulin	412	-1.10	0.06
Androstanediol Glucuronide	407	0.20	0.06
High 3-α diol G profile			
Testosterone	327	-0.02	0.04
17-β Estradiol	326	-0.67	0.07
Sex Hormone Binding Globulin	324	-0.08	0.05
Androstanediol Glucuronide	324	0.78	0.06
High T, E, and SHBG profile			
Testosterone	484	1.00	0.05
17-β Estradiol	480	0.68	0.05
Sex Hormone Binding Globulin	480	0.53	0.04
Androstanediol Glucuronide	476	0.15	0.05
Low T, E, and 3-α diol G			
Testosterone	298	-0.79	0.07
17-β Estradiol	298	-0.71	0.10
Sex Hormone Binding Globulin	298	0.25	0.07
Androstanediol Glucuronide	295	-0.98	0.04

Abbreviations: **U.S.** NHANES III, United States National Health and Nutrition Examination Survey III; SD, Mean standard deviation; N/A, not applicable

[a] Absolute difference between population total mean value and the individual hormone profile values

Table 3. Hormone profile associations with age, race/ethnicity, and body mass index (kg/m^2) in reduced multinomial regression model[a, b, c], **U.S.** NHANES III

Demographic characteristics	High 3-α diol G		High T, E, SHBG		Low T, E, 3-α diol G	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Age (years)						
17-29	0.4 [d]	(0.2-0.7)	0.4 [d]	(0.3-0.6)	0.3 [d]	(<0.1-1.0)
30-49[a]	1.0	-	1.0	-	1.0	-
50-69	1.9	(0.9-4.1)	2.3 [d]	(1.3-4.2)	11.5 [d]	(4.7-27.7)
70 and over	2.2 [d]	(1.0-4.7)	4.2 [d]	(1.9-8.9)	24.3 [d]	(7.7-76.8)
Race/ethnicity group						
non-Hispanic White[a]	1.0	-	1.0	-	1.0	-
non-Hispanic Black	0.4 [d]	(0.2-0.8)	1.0	(0.5-1.9)	0.7	(0.4-1.4)
Mexican American	1.5	(0.7-3.4)	1.4	(0.8-2.7)	3.1 [d]	(1.7-5.7)
Other	0.8	(0.3-2.2)	0.4 [d]	(0.2-0.8)	1.8	(0.7-4.4)
Body Mass Index (kg/m^2)						
<18.5	2.1	(0.8-5.3)	1.9	(0.2-24.6)	1.0	(0.1-13.6)
18.5-24.9[a]	1.0	N/A	1.0	N/A	1.0	-
25-29.9	0.6	(0.3-1.1)	0.3 [d]	(0.2-0.5)	0.4 [d]	(0.2-0.7)
≥30	0.2 [d]	(0.1-0.4)	0.05 [d]	(0.03-0.1)	0.1 [d]	(0.1-0.2)

Abbreviations: **U.S.** NHANES III, National Health and Nutrition Examination Survey III; 95% CI, 95% confidence interval; OR, odds ratio; BMI, Body mass index.

[a] Low SHBG profile is the reference group for multinomial logistic regression models

[b] The reduced model is adjusted for: age, race/ethnicity, BMI, exam day of the week, fasting in hours, liver enzyme levels, exercise level, smoking status, total calories, total fat, monosaturated fats, polyunsaturated fats, saturated fat, fiber, lycopene, and zinc intake

[c] Models used appropriate strata and weighting for national representation

[d] Statistically significant, *P<0.05*

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CONFLICTS OF INTEREST

The authors have no competing interests.

LICENCE

CONTRIBUTORSHIP STATEMENT

Each Author contributed to the study design, data analysis, and drafting of the final manuscript.

DATA SHARING STATEMENT

NHANES is publicly available data

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TITLE

A Cross-Sectional Study of the Association of Age, Race and Ethnicity, and Body Mass Index with Sex Steroid Hormone Marker Profiles among Men in the National Health and Nutrition Examination Survey (NHANES III)

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ABSTRACT

Objectives: Since sex hormone markers are metabolically linked, **examining sex steroid hormones singly may account for inconsistent findings by age, race/ethnicity, and body mass index across studies**. **First, these markers were statistically combined into profiles to account for the metabolic relationship between markers. Then, the relationships between sex steroid hormone profiles and age, race/ethnicity and body mass index were explored** in multinomial logistic regression models.

Design: Cross-sectional survey

Setting: United States **(U.S.)** Third National Health and Nutrition Examination Survey (NHANES III)

Participants: 1,538 men, >17 years

Primary outcome measure: sex hormone profiles

Results: Cluster analysis was used to identify four statistically determined profiles with Blom-transformed T, E, SHBG, and 3-α diol G. We used these four profiles with multinomial logistic regression models to examine differences by race/ethnicity, age and BMI. Mexican American men >50 years were associated with the profile that had lowest T, E, and 3-α diol G levels compared to other profiles ($P<0.05$). Non-Hispanic Black, overweight (25-29.9 kg/m2), and obese (>30 kg/m2) men were most likely to be associated with the cluster with the lowest SHBG ($p<0.05$).

Conclusion: The associations of **sex steroid** hormone profiles by race/ethnicity are novel, while the findings by age and BMI groups are largely consistent with **observations** from single

hormone studies. Future studies should **validate these hormone profile groups and investigate these profiles** in relation to **chronic diseases and certain cancers**.

Trial registration: N/A

SUMMARY BOX

What is already known on this subject?

Past studies examining sex steroid hormones and their markers have been investigated in an effort to explain disparities in chronic disease **and cancer** rates by race/ethnicity. Epidemiologic studies using single hormones in linear models have been mixed. **Presently,** it is still largely unclear if sex steroid hormones contribute to the racial disparities observed in chronic disease **and cancer** rates.

What does this study add?

Examining hormones in relation to race/ethnicity singly in linear models as done in past studies does not account for the metabolic linkage between sex hormones and their markers. To account for metabolic linkage of sex hormones and markers, we created statistically determined hormone profiles by using cluster analysis. Then, we used these profiles as outcome variables in multinomial logistic regression models to determine if there were differences by age, BMI and race/ethnicity groups. Older men and Mexican American men were more likely to be associated with the profile that had lowest T, E, and 3-α diol G levels compared to other profiles ($P<0.05$), and Non-Hispanic Black, overweight (25-29.9 kg/m^2), and obese ($\geq$30 kg/m^2) men were most likely to be associated with the cluster with the lowest SHBG ($P<0.05$). **Findings by race/ethnicity groups and sex steroid hormones are novel compared to using linear models, while findings by age and BMI are largely consistent with single hormone studies.** Using statistically determined hormone marker profiles rather than hormones singly in models **may be a better approach to use to explain** the relationship between sex steroid hormones and race/ethnicity **and other potential risk factors for disease.**

INTRODUCTION

Sex steroid hormones, testosterone (T) and 17-β estradiol (E), along with sex hormone binding globulin (SHBG), a carrier protein of T and E, and androstanediol glucuronide (3-α diol G) a metabolite used as a marker for T and dihydrotestosterone (DHT) metabolism, play critical roles in sexual development and body function [1-5]. These hormone markers are involved in muscle and bone growth, adipose tissue function and distribution [6-8]. **Differences in the levels of sex hormone markers have been hypothesized to contribute to differences in several chronic diseases and prostate cancer rates observed by age, race/ethnicity, and BMI [9-20]. Yet, differences in sex steroid hormone marker levels by age, race/ethnicity, and BMI groups have yet to be fully clarified in the literature [21-35].**

Many previous studies have investigated **single sex** hormone marker levels **in linear regression models** by age, race/ethnicity, and BMI. Typically, with increasing age, T and E levels decline, and SHBG increases, although there **was** evidence to suggest that some older men have hormone marker levels similar to younger men [19, 21-22, 26, 28-29, 35-39]. **By race/ethnicity, higher hormone levels have been reported among non-Hispanic Black men compared to non-Hispanic Whites, although this finding was not consistent across studies; and, studies sex hormone markers among other racial/ethnic groups were scant** [5, 8, 30, 34-35, 40-42]. With increasing BMI, T has been reported to decline and E and SHBG increase, yet these findings are not consistent across studies [8, 21, 24, 26-28, 40]. **Based on somewhat inconsistent findings across these studies, it is possible that investigating factors that influence sex steroid hormone marker levels singly was inadequate.**

Sex hormone markers E, T, SHBG, and 3-α diol G are known to be related through sex steroid metabolism. **Since sex hormone markers are related, then differing hormone levels may be related to each other as well. Cluster analysis can identify underlying statistical patterns among sex hormone markers, which may be indicative of general patterns of sex steroid hormone markers among men. Investigating statistically related sex steroid hormone profiles** may **produce different associations** with **age, race/ethnicity, and BMI groups** than investigating these markers **singly in linear models**. Therefore, we used cluster analysis to statistically determine which mean hormone marker levels cluster together to form specific hormone profiles, and multinomial logistic regression to determine whether age, BMI, and race/ethnicity groups **were** more likely to be associated with different sex steroid hormone marker profiles.

MATERIALS AND METHODS

Study population

We utilized data from the National Health and Nutrition Examination Survey (NHANES) III conducted by the National Center for Health Statistics (NCHS), and these methods have been described previously [43-44]. Briefly, NHANES III **was** collected in two phases, and this study used the phase I data from 1988-1991. This cross-sectional survey was designed as a multistage stratified, clustered probability sample, the sampling frame includes **U.S.** residents ≥2 month**s** of age, civilian, non-institutionalized population, and NHANES III over sampled those >65 years, Non-Hispanic Blacks and Mexican Americans.

The NHANES III study population was used to derive the analysis cohort. A total of 16,295 men were interviewed of which n=14,781 completed a mobile examination component (MEC)

exam [43-44]. The NHANES III morning portion of the survey phase I (1988-1991), included n=2,417 men and n=1,637 **that provided blood** samples. We removed the males that were under 17 years of age and **four outliers with high 17-β estradiol levels identified by box and whisker plot analysis** for a final analysis cohort of n=1,528 men.

Exposure variables

Age, race/ethnicity and BMI **were** the exposures of interest. The NHANES III data obtained age and race/ethnicity information from the **U.S.** Census survey 1990 to draw the sampling frame, so this information **was** 100% complete and was verified during the adult interview survey screening by NHANES III field staff [43-44]. **Continuous age (in years) was categorized into the following groups: 17-29, 30-49, 50-69, and 70 and over. Race/ethnicity was categorized as White non-Hispanic, Black non-Hispanic, Mexican Americans and All others. Asian, American Indian/Alaskan Native, or Pacific Islanders were included in the other group. Whites and Blacks in the analysis reported non-Hispanic ethnicity. Mexican American is considered an ethnicity, and may also report any race group (White, Black, Asian, American Indian/Alaskan Native, or Pacific Islander). Hispanics other than Mexican Americans were included in the other group, since there were few.** BMI (weight in kg divided by height in m squared) was obtained from body measurements taken during the MEC. BMI information is available for 99.5% (n=1,524) of men in the analysis cohort. Categories **of BMI were constructed** based on World Health Organization (WHO) guidelines: underweight <18.5, normal weight 18.5-24.9, overweight 25.0-29.9, and obese $\geq$30 kg/m^2 [45].

Outcome variable

Laboratory measurement methods in NHANES III have been described previously [43-44]. Briefly, NHANES III selected a random subset of n=1,637 men over 12 years of age during the 1988-1991 phase I survey collection, where morning blood samples were collected to measure serum levels of T, E, SHBG, and 3-α diol G using standard procedures. As described previously, samples were centrifuged, serum was aliquotted and stored at -70 C°. Samples were randomly ordered and technicians were blinded to identity, age, and race/ethnicity. The lowest detection limits by the electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer for the samples were: T 0.02 ng/ml, E 5.0 pg/mL, and SHBG 0.35 nmol/L. Enzyme immunoassays were used for 3-α diol G and the lowest detection limits were 0.33 ng/mL. The functional sensitivity, or the lowest analyte concentration that can be reproduced with a coefficient of variation $\geq$20% for T **was** 0.12 ng/mL and 12 pg/mL for E. Control samples were run at the start of the day, after every 100 samples, at the end of the day, once per reagent kit and after calibration. Control samples fell within 2 standard deviations (SD) at the start of the sample runs, but 3 SD **were** tolerated at prior control points. Hormone marker data **were** included as continuous variables in the NHANES III dataset.

Blom-transformations of the laboratory results for T, E, SHBG, and 3-α diol G were used in this analysis. Blom-transformed hormone marker variables were chosen for cluster analysis since these are rank approximations, and were unit-free, which makes the distribution of the four markers comparable [46]. The Blom-transformed marker variables were moderately correlated ($r_{Spearman}$ <0.50) indicating that the unweighted observations are independent, and can be used for cluster analysis.

Model covariates

To adjust the regression models for lifestyle and dietary factors, we used data from the NHANES III adult, examination, and laboratory files. Alcohol intake, smoking status, exercise amount, zinc, total calorie, total fat, total monosaturated fat, total polysaturated fat, total saturated fat, fiber, were taken from 24-hour recall surveys, which captured food intake from the past twenty four hours [43-44]. Lycopene intake was from blood samples since it was not available from 24-hour recall surveys. Alcohol intake (grams) was combined into three groups (**n**on-drinkers, drinkers, and missing). Smoking status **was** categorized into four levels, as men who do not smoke, men who smoke, but not every day, and men who are current everyday smokers of <35 cigarettes per day **or** ≥35 cigarettes per day. The exercise variable combined the total days per month a person participated in **exercise activities**. Serum lycopene concentration was measured in blood samples, and if levels were below detection (0.63 µg/ml) 0 was recorded. Exercise per month, lycopene concentrations, other food intake variables were grouped into quartiles.

The medical exam variables used in the models, included fasting status, exam day of the week, blood cholesterol level, aspartate aminotransferase, and alanine aminotransferase were from the MEC data. Fasting compliance was determined prior to blood and urine collection via questionnaire, and was not followed uniformly, for instance: <1% fasted for 20 hours or more, 91.8% fasted for 10.01-19.99 hours, 7.5% fasted for 10 hours or less, and <0.1% either did not fast or no value was available. No minimum detection limits were presented for cholesterol, aspartate aminotransferase and alanine aminotransferase. Cholesterol, aspartate aminotransferase and alanine aminotransferase were categorized into quartiles for analysis.

Data analysis

All data analysis was conducted using SAS 9.2 (Cary, NC). K-means cluster analysis was chosen to create cluster profiles using Blom-transformed T, E, SHBG, and 3-α diol G over other exploratory methods, since it assigns each observation only to one group, **is** based on least squares, tends to find clusters with roughly the same number of observations, and is robust to outliers in the data. The k-means procedure calculates statistics that can be used to determine the best number of k clusters, including: an approximate overall R-squared value, Pseudo F-Stat, and Cubic Clustering Criteria (CCC). These statistics were employed to compare exploratory cluster solutions using 4 to 8 cluster groups on the unweighted data**, since survey procedures were not available for cluster analysis in SAS 9.2**.

Multinomial logistic regression models using survey procedures **(accounting for weighted and stratified data)** were employed to examine how age, race/ethnicity, and BMI were associated with **the constructed** sex hormone profiles. Low SHBG served as the reference group since mean hormone values were most similar to the total population. Models were reduced by investigating the exposure variables (age, race/ethnicity, and BMI) for a 10% change in the **odds ratios** (ORs). Covariates included in the full models included age, race/ethnicity, BMI, exam day of the week, hours of fasting, aspartate aminotransferase, alanine aminotransferase, cholesterol levels, exercise level, smoking and drinking status, total calories, total fat, monosaturated fats, polyunsaturated fats, saturated fat, fiber, lycopene, and zinc intake.

RESULTS

We calculated the percentages (%) and 95% confidence intervals (CIs) for age, race/ethnicity, and BMI (n=1,528) (table 1). The majority of men in the cohort **were** 30-49 yrs. (42.25%),

followed by 17-29 yrs. (29.05%), 50-69 yrs (21.18%), and over 70 yrs (7.52%). By race/ethnicity, men self-reported to be, non-Hispanic White (77.36%), and non-Hispanic Blacks (9.75%), Mexican American (5.25%), and all other races (7.64%). The highest proportions of men were either overweight, BMI 25.0-29.9, (39.73%) or normal weight, BMI 18.5-24.9 (38.45%), while 20.38% of men were considered obese, BMI $\geq$30.

We used cluster analysis to create hormone profiles from Blom-transformed T, E, SHBG, and 3-α diol G laboratory values, and only the four and five level cluster solutions performed well (data not shown). The pseudo F-statistic was improved over the five cluster solution, and the CCC value was positive (1.2) for the four cluster solution (data not shown). The four cluster solution was used to create hormone profiles (table 2).

We examined the mean levels of Blom-transformed T, E, SHBG, and 3-α diol G for the hormone profiles and the total population to determine how the mean levels differed (table 2). The first cluster had lowest mean SHBG level of the groups, but the mean level of T, E, and 3-α diol G was the most similar to the total cohort (hereafter referred to as the 'low SHBG profile'). The second cluster had the highest mean 3-α diol G level compared to the other clusters (referred to as the 'high 3-α diol G profile'). The third cluster had the highest mean levels of T, E and SHBG (hereafter referred to as the 'high T, E, and SHBG profile'). The fourth cluster had lowest mean levels of T, E, and 3-α diol G compared to the other groups ('low T, E, and 3-α diol G profile').

Associations with hormone profiles and age, race/ethnicity, and BMI groups using **weighted** multinomial logistic regression models were examined (table 3). The younger men (17-29 yrs) were associated with the 'low SHBG profile'. Men in the 'low T, E, and 3-α diol G

profile' were most associated with 50-69 yrs (OR =11.5, 95% confidence interval (CI): 4.74, 27.68) and 70 yrs or over (OR=24.3, 95% CI: 7.71, 76.82). Non-Hispanic Black men had higher odds of being in the 'low SHBG profile' (OR=2.5, 95% CI: 1.30, 4.35), and Mexican American men were more strongly associated with the 'low T, E, and 3-α diol G profile' (OR=3.1, 95% CI: 1.69, 5.68). **Obese men (BMI ≥30) were most likely to be associated with the referent 'low SHBG profile' compared to men with a normal BMI (18.5-24.9) in all other profiles.**

DISCUSSION

This is the first study to examine statistically determined sex steroid hormone marker profiles by age, BMI, and race/ethnicity groups. Applying our novel approach to studying sex steroid hormone levels among **U.S.** men, we created four statistically determined clusters, described as: 'low SHBG', 'high 3-α diol G', 'high T, E, and SHBG', and 'low T, E, and 3-α diol G'. **Examining hormone profiles by age and BMI, our results largely agree with single hormone studies [5, 16, 21, 24, 30, 40, 47]. This study also found new evidence supporting differences in sex steroid hormone levels for non-Hispanic Blacks and Mexican American men using hormone profiles, and these observations differed from single hormone studies.**

Men in our study associated with the 'low SHBG' profile were more likely to be younger (<17-29 yrs), obese (BMI ≥30), and non-Hispanic Black (table 3). Our findings indicate that the 'low SHBG profile' **was** more commonly associated with younger men (17-29 yrs) [5, 16, 30, 40], and lower SHBG levels were reported among obese men in single hormone studies [21, 24, 47]. By contrast, the **observations** that non-Hispanic Blacks **were** more likely to be associated with a 'low SHBG profile' compared to non-Hispanic Whites and Mexicans **were new**. This result does not agree with previous single hormone studies, which have **dominantly** reported no

differences or higher levels of SHBG among non-Hispanic Blacks compared to non-Hispanic Whites [5, 16, 30-31, 40].

The 'high 3-α diol G profile' associations with age and BMI **were** somewhat ambiguous compared to other profiles, while the 'high 3-α diol G profile' **was** more strongly associated with non-Hispanic Whites. Past studies investigating 3-α diol G have reported higher 3-α diol G activity among older men with a higher BMI [8, 32-33, 41-42, 48-53]. However, much stronger associations with older age and obesity **were** seen with other profiles compared to this profile [8, 32-33, 41-42, 48-53]. **Some single hormone studies reported no difference in 3-α diol G levels by race among younger men [41], yet in other studies older men have reported higher 3-α diol G activity in non-Hispanic Whites compared to non-Hispanic Blacks which agrees with the findings in this study [5, 41-42, 50-51].** The reasons why hormone studies **were** largely consistent for higher 3-α diol G levels seen among older non-Hispanic White men, while findings for other race/ethnicity groups **were** inconsistent **was** still unclear [5, 9, 16, 30-31, 34, 40, 42, 50].

The men in the 'high T, E, and SHBG profile' **were** older than the first two profiles, **were** most likely to have a normal BMI, and there **were** not any differences between the race/ethnicity groups. Previous cross-sectional studies investigating T alone have reported high T levels among young men, yet other studies have indicated that high T levels **were** not found exclusively among young men [19, 22, 26, 36-39, 54-57]. The results for the 'high T, E, and SHBG' profile **were** consistent with single hormone studies that reported higher T and SHBG among men with a normal BMI [58-59]. **Past studies have hypothesized that higher sex steroid hormones (T and E) were responsible for the racial disparities observed in the rates of prostate cancer [9, 22, 30, 35, 40]. Despite a higher proportion of non-Hispanic Black men found in the**

'high T, E, and SHBG profile', non-Hispanic Black men were not associated with this profile (data not shown). These findings do not support this previously considered hypothesis that sex steroid hormone levels are higher among non-Hispanic Black men compared to other race/ethnicity groups [5, 9, 22, 30, 35, 40, 46].

The 'low T, E, and 3-α diol G profile' **was** more likely to be associated with men over 70 yrs and Mexican American men, while findings by BMI **were** less defined compared to other profiles. Previous studies have suggested that lowered T and E metabolism, and increasing SHBG levels **were** associated with older ages [19, 22, 26, 36-39, 54]. This **was** in agreement with our results, since 74% of men are over 50 yrs in this profile, and associations **were** strongest with older age groups (table 3). Overweight and obesity have been associated with declines in T and SHBG, and despite the low T levels in this profile, the 'low SHBG profile' was more strongly associated with obesity [24, 58-59]. Past single hormone studies specifically comparing T levels among Hispanics to non-Hispanics have conflicted, two reported no differences, one reported lower, and another reported higher levels [5, 9, 42, 46]. Although there were few studies comparing sex steroid hormones among Mexican Americans compared to non-Hispanic Whites **these findings from these studies conflict [5, 9, 16, 30-31, 40, 42].**

This study has several strengths. NHANES III data **was** a nationally representative sample, so selection bias **was** minimized [43-44]. The NHANES III oversampled minorities and men over 65 years ensuring adequate numbers of men for analysis [43-44]. Our exposure variables were 99% complete [43-44]. Hormone levels were measured systematically using standard methods available at the time which did employ testing against control samples [43-44]. We were able to select only those men that provided **blood** samples in the morning to correct for

daily hormone fluctuations, and control for day of the week the blood was drawn and fasting time [43-44].

The study also has several limitations. **The dietary information was from self-reported 24-hour recall surveys, and may not reflect a man's true dietary behaviors** [43-44]. Smoking status was also self-reported [43-44]. The study was only based on a single hormone measurement among men, which may not account for the daily complexity or serum hormone measurements over time. While the hormone profiles combine two major sex steroid hormones, a carrier protein and a metabolite, these profiles are still likely to be an oversimplification **compared to hormone metabolism in the body**.

In conclusion, specific sex steroid hormone marker profiles were more likely than others to be associated with one or more age, race/ethnicity, or BMI groups. Our findings for non-Hispanic Blacks and Mexican Americans are novel, since these groups have often been suggested to be associated with higher sex steroid hormone levels, yet this study found the opposite. Our findings by age and BMI largely agreed with most single hormone studies. **The observed race/ethnicity differences across the hormone profiles in the current analysis, suggest that when accounting for the relationship between sex steroid hormone markers, race/ethnicity differences become apparent. Further research is necessary to determine if sex steroid hormone profiles contribute to the increased risk of several cancers and chronic diseases observed by race/ethnicity.**

Table 1. Demographic information among men, **U.S.** NHANES III 1988-1991

Demographic information	Total (n)	Percentage (%)
Age		
17-29	365	29.1
30-49	516	37.3
50-69	388	21.2
70 and over	259	7.5
Race/ethnicity		
Non-Hispanic White	689	77.4
Non-Hispanic Black	378	9.8
Mexican American	402	5.3
Other	59	7.6
Body Mass Index (kg/m^2)		
<18.5	21	1.4
18.5-24.9	555	38.5
25.0-29.9	623	39.7
≥30	328	20.4
Missing	1	<0.01

Abbreviations: **U.S.** NHANES III, United States National Health and Nutrition Examination Survey III

Table 2. Blom-transformed sex steroid hormone marker mean levels in the total population and hormone marker profiles among American men, **U.S.** NHANES III 1988-1991

Hormone Marker Group	Total (N)	Mean	SD
Population Total			
Testosterone	1527	0.13	0.04
17-β Estradiol	1524	0.06	0.06
Sex Hormone Binding Globulin	1517	-0.15	0.05
Androstanediol Glucuronide	1505	0.16	0.04
Low SHBG profile			
Testosterone	415	-0.25	0.04
17-β Estradiol	417	0.32	0.06
Sex Hormone Binding Globulin	412	-1.10	0.06
Androstanediol Glucuronide	407	0.20	0.06
High 3-α diol G profile			
Testosterone	327	-0.02	0.04
17-β Estradiol	326	-0.67	0.07
Sex Hormone Binding Globulin	324	-0.08	0.05
Androstanediol Glucuronide	324	0.78	0.06
High T, E, and SHBG profile			
Testosterone	484	1.00	0.05
17-β Estradiol	480	0.68	0.05
Sex Hormone Binding Globulin	480	0.53	0.04
Androstanediol Glucuronide	476	0.15	0.05
Low T, E, and 3-α diol G			
Testosterone	298	-0.79	0.07
17-β Estradiol	298	-0.71	0.10
Sex Hormone Binding Globulin	298	0.25	0.07
Androstanediol Glucuronide	295	-0.98	0.04

Abbreviations: **U.S.** NHANES III, United States National Health and Nutrition Examination Survey III; SD, Mean standard deviation; N/A, not applicable

[a] Absolute difference between population total mean value and the individual hormone profile values

Table 3. Hormone profile associations with age, race/ethnicity, and body mass index (kg/m^2) in reduced multinomial regression model[a, b, c], **U.S.** NHANES III

Demographic characteristics	High 3-α diol G		High T, E, SHBG		Low T, E, 3-α diol G	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Age (years)						
17-29	0.4 [d]	(0.2-0.7)	0.4 [d]	(0.3-0.6)	0.3 [d]	(<0.1-1.0)
30-49[a]	1.0	-	1.0	-	1.0	-
50-69	1.9	(0.9-4.1)	2.3 [d]	(1.3-4.2)	11.5 [d]	(4.7-27.7)
70 and over	2.2 [d]	(1.0-4.7)	4.2 [d]	(1.9-8.9)	24.3 [d]	(7.7-76.8)
Race/ethnicity group						
non-Hispanic White[a]	1.0	-	1.0	-	1.0	-
non-Hispanic Black	0.4 [d]	(0.2-0.8)	1.0	(0.5-1.9)	0.7	(0.4-1.4)
Mexican American	1.5	(0.7-3.4)	1.4	(0.8-2.7)	3.1 [d]	(1.7-5.7)
Other	0.8	(0.3-2.2)	0.4 [d]	(0.2-0.8)	1.8	(0.7-4.4)
Body Mass Index (kg/m^2)						
<18.5	2.1	(0.8-5.3)	1.9	(0.2-24.6)	1.0	(0.1-13.6)
18.5-24.9[a]	1.0	N/A	1.0	N/A	1.0	-
25-29.9	0.6	(0.3-1.1)	0.3 [d]	(0.2-0.5)	0.4 [d]	(0.2-0.7)
≥30	0.2 [d]	(0.1-0.4)	0.05 [d]	(0.03-0.1)	0.1 [d]	(0.1-0.2)

Abbreviations: **U.S.** NHANES III, National Health and Nutrition Examination Survey III; 95% CI, 95% confidence interval; OR, odds ratio; BMI, Body mass index.

[a] Low SHBG profile is the reference group for multinomial logistic regression models

[b] The reduced model is adjusted for: age, race/ethnicity, BMI, exam day of the week, fasting in hours, liver enzyme levels, exercise level, smoking status, total calories, total fat, monosaturated fats, polyunsaturated fats, saturated fat, fiber, lycopene, and zinc intake

[c] Models used appropriate strata and weighting for national representation

[d] Statistically significant, *P<0.05*

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Each Author contributed to the study design, data analysis, and drafting of the final manuscript.

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