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ORIGINAL PAPER

Body fatness and sex steroid hormone concentrations in US men: results from NHANES III

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Abstract
Objective Obesity is associated with a variety of chronic diseases, including cancer, which may partly be explained by its influence on sex steroid hormone concentrations. Whether different measures of obesity, i.e., body mass index (BMI), waist circumference, and percent body fat were differentially associated with circulating levels of sex steroid hormones was examined in 1,265 men, aged 20–90+ years old, attending the morning examination session of the Third National Health and Nutrition Examination Survey (NHANES III).
Materials and methods Serum hormones were measured by immunoassay. Weight, height, and waist circumference were measured by trained staff. Percent body fat was estimated from bioelectrical impedance. Multivariate linear regression was used to estimate associations between body fatness measures and hormone levels.
Results Total and free testosterone and sex hormone binding globulin concentrations decreased, whereas total and free estradiol increased with increasing BMI, waist circumference, and percent body fat (all p trend < 0.05). The magnitude of change in these hormones was similar for a one-quartile increase in each body fatness measure.
Conclusion Measured BMI, waist circumference, and percent body fat led to similar inferences about their association with hormone levels in men.

Keywords NHANES III · Testosterone · Estradiol · Obesity

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Introduction

Obesity is a growing problem in Western countries, including the United States, and an emerging problem in Asian countries [1]. Obesity leads to health consequences, including a higher risk of type 2 diabetes mellitus, heart disease, cancer [2, 3], and premature death overall and from cardiovascular disease and cancer [4]. The precise mechanisms by which obesity influences chronic disease risk are not entirely clear yet, but one line of evidence involves changes in circulating levels of sex steroid hormones and sex hormone binding globulin (SHBG) with obesity, particularly with respect to cancer [5]. More body fat leads to a higher conversion of testosterone to estradiol by aromatase in fat tissue [6]. This increased conversion suppresses luteinizing hormone release [7, 8], which results in a reduced production of testosterone by the Leydig cells. Estrogens also inhibit the activity of 17-α-hydroxylase and 17, 20 lyase, thus, inhibiting intratesticular steroidogenesis [9]. High insulin levels in obese men may inhibit hepatic SHBG production in HepG2 cells [10].

Most studies that examined the association between obesity and circulating sex steroid hormone levels have relied on either body mass index (BMI) or waist circumference (or waist-to-hip ratio) as indicators of body fatness. The association between percentage of body fat and circulating steroid hormone levels has been less well studied. Although these three measures are correlated, they do reflect different aspects of obesity [11]. Thus, the aims of this study were (a) to estimate the associations between three measures of body fatness with serum sex steroid concentrations overall and after taking into account factors that are both associated with hormones and body fatness, and (b) to assess differences in the magnitude of the associations between different measures of body fatness and hormones in a nationally representative sample of US men.

Materials and methods

Study population

The Third National Health and Nutrition Examination Survey (NHANES III) is a cross-sectional study that was conducted by the National Center for Health Statistics between 1988 and 1994. It is based on a multistage stratified, clustered probability sample of the US civilian non-institutionalized population at least 2 months old [12]. Specific sub-groups of the US population, including Mexican–Americans, non-Hispanic blacks, and the elderly, were over-sampled to ensure minimum sample sizes. NHANES III was conducted in two phases (1988–1991 and 1991–1994) and unbiased national estimates of health and nutrition characteristics can be independently produced for each of these two phases. Within each phase, subjects were randomly assigned to participate in either the morning or afternoon/evening examination session. More than 33,000 subjects participated in NHANES III. Of these, 1,998 men at least 20 years of age participated in the morning session of phase I. Morning sample participants were chosen for this hormone study to reduce extraneous variation due to diurnal production of hormones. For the purpose of our study, serum samples for hormone measurements were still available for 1,470 of these men. Men with a history of prostate cancer were excluded because certain treatments may have affected hormone levels ($n = 12$). We further excluded 193 men due to missing information on BMI ($n = 1$), waist circumference ($n = 47$), percent body fat ($n = 94$), and other covariates ($n = 6$). Sixteen men were excluded for having missing hormone measurements and an additional 29 men were excluded for having extreme hormone measurements. The following cut-points were used to determine extreme hormone measurements and are based on visual inspection of the distribution of each hormone on the natural log scale: ln(testosterone) ≤ 0.4 (1.5% ile), ln (free testosterone) ≤ -4.0 (1.4% ile), ln (free estradiol) ≤ -1.5 (0.15% ile), ln(SHBG) ≤ 1.7 (0.08% ile), and ln (AAG) ≤ 2.0 (0.5% ile), leaving 1,265 men for the final analyses.

Subjects participated in an interview that was conducted at home and in an extensive physical examination, which included a blood sample collection. Cigarette smoking, alcohol consumption, and physical activity were assessed using a questionnaire. Height was measured to the nearest 0.1 cm using a stadiometer and weight was measured to the nearest 0.01 kg using an electronic digital scale while the participant was wearing foam slippers and paper shirt and pants. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the iliac crest to the nearest 0.1 cm. A Valhalla Scientific Body Composition Analyzer (model 1990B; Valhalla Scientific, Inc., San Diego, CA) was used to measure whole body electrical resistance. Validated prediction equations using height and weight were used to convert whole body electrical resistance at 50 kHz to percent body fat [13].

Measurement of serum sex steroid hormones and SHBG

Blood was drawn after an overnight fast for participants in the morning sample during either an examination at a medical examination center or during an abbreviated examination at home. After centrifugation, serum was aliquoted and stored at −70°C until they pulled from the freezers for this

project. The serum samples were shipped on dry ice directly from the National Center for Health Statistics' main repository in Atlanta, GA, to the assay laboratory.

Serum hormone concentrations were measured in the laboratory of Dr. Nader Rifai at Children's Hospital in Boston, MA. Competitive electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN) were used to quantify serum testosterone, estradiol, and SHBG. Androstanediol glucuronide was measured by an enzyme immunoassay (Diagnostic Systems Laboratories, Webster, TX). The participant samples were randomly ordered for testing and the laboratory technicians were blinded to the identities and characteristics of the participants. The lowest detection limits of the assays were: testosterone 0.02 ng/ml, estradiol 5 pg/ml, androstanediol glucuronide 0.33 ng/ml, and SHBG 3 nmol/l. The coefficients of variation for quality control specimens included during the analyses of the NHANES III specimens were as follows: testosterone 5.9 and 5.8% at 2.5 and 5.5 ng/ml; estradiol 6.5 and 6.7% at 102.7 and 474.1 pg/ml; androstanediol glucuronide 9.5 and 5.0% at 2.9 and 10.1 ng/ml; and SHBG 5.3 and 5.9% at 5.3 and 16.6 nmol/l. In addition, we ran quality control samples with a mean estradiol concentration of 39.4 pg/ml, which is in the range of typical male estradiol concentrations; the intra-assay CV% was 5.2% and the inter-assay CV% was 2.5%. Free testosterone concentration was estimated from measured total testosterone, SHBG, and albumin (already available in the NHANES III public use database) [14]; free estradiol was calculated from total estradiol, SHBG, and albumin [15].

Statistical analysis

All statistical analyses were performed using SUDAAN [16] as implemented in SAS v.9.1 (Cary, NC). Sampling weights were applied to take into account selection probabilities, over-sampling, non-response, and differences between the sample and the total US population [12].

We computed the Spearman correlation coefficient adjusted for age between the different hormones. Linear regression models were used to estimate the association between quartiles of the three measures of body fatness—BMI, waist circumference, and percent body fat—and hormone concentrations. Because hormone and SHBG concentrations were not normally distributed, we used log-transformed data. In Model 1, we adjusted for age (1 year increments) and race/ethnicity (non-Hispanic black, non-Hispanic white, Mexican–American, other). To evaluate the possibly confounding effects of factors that influence hormone concentrations, in Model 2, we included age and race/ethnicity as well as cigarette smoking (never smoker, former smoker, current smoker), alcohol consumption (never drinker, $\leq$1 drink/week, >1 drink/week to <1 drink/day, 1+ drink/day), and physical activity (moderate or vigorous physical activity on 0 times/week, <3 times/week, $\geq$3 to <8 times/week, >8 times/week). When Model 2 was further adjusted for serum cotinine and a spline term for age at 42 years, the inferences did not significantly change; therefore, we did not include these factors in the final Model 2. In Model 3, we adjusted for the factors in Model 2 plus mutually adjusted testosterone, estradiol, and SHBG because these hormones compete for binding with SHBG, and adjusted free testosterone for total estradiol and free estradiol for total testosterone. We compared geometric mean concentrations of hormones and SHBG across quartiles of BMI, waist circumference, and percent body fat using analysis of variance. We estimated the slope of the change in the natural logarithm hormone concentration with increasing body fatness by entering into the models an ordinal variable with values of 1, 2, 3, and 4 corresponding to quartile of each body fatness measure; we tested its coefficient using the Wald test. We used quartiles to compare the strengths of the association using a comparable scaling of each body fat measure; one unit change in BMI, waist circumference, and percent body fat are not comparable in scale, but taking the distribution of these fat measures, which each captures extent of body fatness, and dividing them based on achieving equal numbers of men should yield approximately comparable scaling.

While each of the three anthropometric measures reflects the underlying extent of body fat, each is measured with error, although the sources of errors may not be the same. Thus, we cross-classified the men based on quartiles of 2 of the 3 body fatness measures at a time and estimated the geometric mean hormone concentrations for men in the lowest quartile on both of the measures, the highest quartile on both of the measures, and all other combinations. We then compared the geometric means for the combinations to the means for the individual measures.

Finally, we computed the changes in BMI, waist circumference, and percent body fat that were associated with a 2% decline in the geometric mean of total testosterone, free testosterone, total estradiol, free estradiol, SHBG, and androstanediol glucuronide. These estimates were calculated based on geometric mean hormone levels for a 50-year-old white man who is a non-smoker, in the second quartile of alcohol consumption and physical activity.

All significance tests were two-sided; $p < 0.05$ was considered to be statistically significant.

The protocols for the conduct of NHANES III were approved by the Institutional Review Board of the National Center for Health Statistics, US Centers for Disease Control and Prevention. Informed consent was obtained from all participants. The measurement of sex steroid hormones in these stored serum specimens were approved by Institutional Review Boards at the Johns Hopkins Bloomberg

School of Public Health and the National Center for Health Statistics, US Centers for Disease Control and Prevention.

Results

The distribution of baseline characteristics in the study population after applying sampling weights is shown in Table 1. The prevalence of current smoking was 34.0 and 17.4% of men consumed at least one alcoholic drink/day. One-third of men had moderate or vigorous activity 8 or more times/week; this included walking or stair climbing. The prevalence of obesity was 17.4% based on BMI ($\geq$30 kg/m^2), 24.5% based on waist circumference ($\geq$102 cm), and 48.8% based on percent body fat ($\geq$25%; see [17]). The partial Spearman correlation coefficients (adjusted for age and race/ethnicity) between the three measures of body fatness were: BMI and waist circumference: $r = 0.93$; BMI and percent body fat: $r = 0.68$; and waist circumference and percent body fat: $r = 0.71$.

Almost all hormones were statistically significantly correlated, although the strength of correlation differed. Correlation coefficients of total testosterone with free testosterone, total and free estradiol, androstanediol glucuronide, and SHBG were 0.75, 0.43, 0.15, 0.11, 0.63, respectively (all p values ≤ 0.0001); correlation coefficients of free testosterone with total and free estradiol, androstanediol glucuronide, and SHBG were 0.49, 0.47, 0.16, 0.06, respectively (all p values ≤ 0.0001; SHBG: $p = 0.046$); correlation coefficients of total estradiol with free estradiol, androstanediol glucuronide, and SHBG were 0.89, 0.09, and 0.11, respectively (all p values ≤ 0.0001; androstanediol glucoronide $p = 0.003$); correlation coefficients of free estradiol with androstanediol glucuronide and SHBG were 0.07 ($p = 0.01$) and -0.27 ($p \leq 0.0001$),

Table 1 Selected characteristics and hormone concentrations of 1,265 adult men who participated in the morning examination session of Phase I of the third national health and nutrition examination survey (NHANES III)

Subject characteristics	Unweighted sample size	Mean or percentage (SE)[a]	IQR
Age (years)	1,265	41.6 (0.7)	27–62
Race/ethnicity (%)			
Non-hispanic white	590	78.6 (3.1)	
Non-hispanic black	298	8.9 (1.3)	
Mexican–American	326	5.0 (0.8)	
Other	51	7.4 (2.1)	
Body fat (%)	1,265	24.9 (0.3)	21.8–29.4
Body mass index (kg/m^2)	1,265	26.3 (0.2)	22.7–28.7
Waist circumference (cm)	1,265	94.4 (0.6)	83.5–102.3
Cigarette smoking (%)			
Never	433	34.5 (2.3)	
Former	424	31.5 (2.9)	
Current	408	34.0 (2.1)	
Alcohol consumption (%)			
0 drinks/month	461	30.6 (2.5)	
0.1–4 drinks/month	224	17.0 (1.6)	
4.1–29.9 drinks/month	372	35.0 (2.1)	
$\geq$30 drinks/month	208	17.4 (2.6)	
Frequency of physical activity (%)			
0 times/week	162	8.0 (1.3)	
0.1–2.9 times/week	388	29.3 (2.0)	
3–7.9 times/week	357	29.8 (1.2)	
$\geq$8 times/week	358	32.9 (2.9)	
Total testosterone (ng/ml)[b]	1,265	5.59 (0.09)	3.8–6.5
Total estradiol (pg/ml)[b]	1,265	37.3 (0.7)	28.6–43.1
SHBG (nmol/L)[b]	1,265	37.7 (0.7)	26.1–51.5
Androstanediol glucuronide (ng/ml)[b]	1,265	14.4 (0.5)	7.0–15.9
Free testosterone (ng/ml)[b]	1,265	0.112 (0.002)	0.07–0.13
Free estradiol (pg/ml)[b]	1,265	0.962 (0.02)	0.71–1.1

SE standard error, *IQR* interquartile range

[a] Sampling weights were applied

[b] Geometric mean

Table 2 Sex steroid hormone and SHBG concentrations by quartiles of BMI in men, NHANES III, 1988–1991

BMI		Model 1[a]		Model 2[b]		Model 3[c]	
Quartile	Range (kg/m^2)	Mean	95% CI	Mean	95% CI	Mean	95% CI
Total testosterone (ng/ml)							
Q1	≤22.9	6.16	(5.96, 6.37)	6.07	(5.85, 6.30)	5.55	(5.36, 5.75)
Q2	23.0–25.3	5.73	(5.49, 5.98)	5.64	(5.40, 5.88)	5.48	(5.33, 5.65)
Q3	25.4–28.7	5.13	(4.91, 5.37)	5.19	(4.96, 5.43)	5.35	(5.19, 5.53)
Q4	>28.7	4.28	(4.12, 4.44)	4.38	(4.23, 4.52)	4.74	(4.58, 4.91)
Slope, *p* trend[d]		−0.12	<0.001	−0.11	<0.001	−0.05	<0.001
Free testosterone (ng/ml)							
Q1	≤22.9	0.11	(0.106, 0.114)	0.108	(0.104, 0.111)	0.109	(0.105, 0.113)
Q2	23.0–25.3	0.111	(0.106, 0.116)	0.109	(0.104, 0.115)	0.11	(0.106, 0.114)
Q3	25.4–28.7	0.106	(0.102, 0.110)	0.107	(0.102, 0.111)	0.108	(0.104, 0.112)
Q4	>28.7	0.097	(0.092, 0.101)	0.099	(0.095, 0.103)	0.096	(0.093, 0.100)
Slope, *p* trend[d]		−0.04	0.001	−0.03	0.02	−0.04	0.002
Total estradiol (pg/ml)							
Q1	≤22.9	35.87	(34.29, 37.53)	35.3	(34.08, 36.57)	34.19	(32.69, 35.77)
Q2	23.0–25.3	36.2	(34.60, 37.87)	35.69	(34.19, 37.27)	34.95	(33.68, 36.28)
Q3	25.4–28.7	34.64	(32.79, 36.59)	35.16	(33.35, 37.07)	35.13	(33.51, 36.82)
Q4	>28.7	37.11	(35.62, 38.67)	37.64	(35.91, 39.45)	39.73	(38.05, 41.48)
Slope, *p* trend[d]		0.006	0.5	0.018	0.1	0.044	<0.001
Free estradiol (pg/ml)							
Q1	≤22.9	0.85	(0.81, 0.90)	0.84	(0.80, 0.87)	0.82	(0.78, 0.85)
Q2	23.0–25.3	0.9	(0.85, 0.95)	0.89	(0.85, 0.94)	0.88	(0.84, 0.93)
Q3	25.4–28.7	0.9	(0.85, 0.95)	0.91	(0.87, 0.96)	0.92	(0.88, 0.96)
Q4	>28.7	1.03	(0.98, 1.08)	1.04	(0.99, 1.10)	1.08	(1.03, 1.13)
Slope, *p* trend[d]		0.056	<0.001	0.066	<0.001	0.086	<0.001
Sex hormone binding globulin (nmol/l)							
Q1	≤22.9	43.38	(40.42, 46.55)	43.29	(40.50, 46.28)	38.59	(36.60, 40.69)
Q2	23.0–25.3	37.45	(35.17, 39.87)	36.89	(34.85, 39.05)	34.95	(33.35, 36.64)
Q3	25.4–28.7	32.52	(30.43, 34.77)	32.62	(30.46, 34.94)	32.88	(31.01, 34.88)
Q4	>28.7	26.71	(25.43, 28.05)	27.06	(25.71, 28.47)	31.75	(30.06, 33.54)
Slope, *p* trend[d]		−0.16	<0.001	−0.15	<0.001	−0.07	<0.001
Androstanediol glucuronide (ng/ml)							
Q1	≤22.9	10.97	(10.02, 12.00)	10.98	(10.03, 12.02)		Not applicable
Q2	23.0–25.3	12.45	(11.16, 13.90)	12.6	(11.34, 14.01)		
Q3	25.4–28.7	12.49	(11.92, 13.09)	12.43	(11.72, 13.18)		
Q4	> 28.7	12.04	(10.93, 13.25)	11.98	(10.86, 13.21)		
Slope, *p* trend[d]		0.028	0.1	0.026	0.2		

[a] *Model 1* adjusted for age and race/ethnicity

[b] *Model 2* adjusted for age and race/ethnicity, smoking, alcohol consumption, physical activity

[c] *Model 3* same as model 2 plus testosterone, estradiol, and SHBG mutually adjusted and free testosterone and free estradiol mutually adjusted

[d] Per 1 quartile change in BMI

respectively; correlation between androstanediol glucuronide and SHBG was 0.0007 (*p* value 0.98).

Total and free testosterone

Total and free testosterone concentrations decreased with increasing extent of each body fatness measure (Tables 2, 3, 4). For total testosterone, the decline in concentration with increasing body fatness was attenuated after further adjustment for smoking, alcohol consumption, and physical activity (Model 2) and was even more greatly attenuated after further adjustment for total estradiol and SHBG (Model 3). For free testosterone, the size of the decline in concentration with increasing body fatness was similar in

Models 1 and 3, but less steep in Model 2. In the fully adjusted model (Model 3), the extent of the decline in total testosterone and also in free testosterone concentrations was similar for a one-quartile change in BMI (Table 2), waist circumference (Table 3), and percent body fat (Table 4).

Total and free estradiol

Total estradiol concentration did not increase with increasing extents of any of the three measures of body fatness after adjustment for age and race/ethnicity (Model 1). However, in the fully adjusted model (Model 3), total

Table 3 Sex steroid hormone and SHBG concentrations by quartiles of waist circumference in men, NHANES III, 1988–1991

Waist circumference		Model 1[a]		Model 2[b]		Model 3[c]	
Quartile	Range (cm)	Mean	95% CI	Mean	95% CI	Mean	95% CI
Total testosterone (ng/ml)							
Q1	≤84.9	6.48	(6.18, 6.79)	6.34	(6.01, 6.69)	5.65	(5.48, 5.83)
Q2	85.0–92.8	5.58	(5.36, 5.82)	5.55	(5.34, 5.77)	5.5	(5.31, 5.69)
Q3	92.9–101.7	5.01	(4.68, 5.35)	5.05	(4.77, 5.34)	5.26	(5.10, 5.43)
Q4	>101.7	4.26	(4.07, 4.47)	4.34	(4.14, 4.56)	4.73	(4.57, 4.90)
Slope, *p* trend[d]		−0.14	<0.001	−0.12	<0.001	−0.06	<0.001
Free testosterone (ng/ml)							
Q1	≤84.9	0.114	(0.110, 0.117)	0.112	(0.108, 0.115)	0.111	(0.107, 0.115)
Q2	85.0–92.8	0.108	(0.104, 0.112)	0.108	(0.103, 0.112)	0.109	(0.105, 0.114)
Q3	92.9–101.7	0.104	(0.098, 0.111)	0.105	(0.099, 0.110)	0.106	(0.102, 0.110)
Q4	>101.7	0.097	(0.092, 0.101)	0.099	(0.094, 0.103)	0.097	(0.093, 0.100)
Slope, *p* trend[d]		−0.05	<0.001	−0.04	0.001	−0.05	<0.001
Total estradiol (pg/ml)							
Q1	≤84.9	36.93	(35.44, 38.48)	36.31	(34.91, 37.76)	34.71	(33.18, 36.31)
Q2	85.0–92.8	35.02	(33.28, 36.85)	34.95	(33.41, 36.56)	34.4	(32.82, 36.05)
Q3	92.9–101.7	34.67	(32.19, 37.36)	35.02	(32.96, 37.22)	35.34	(33.72, 37.04)
Q4	>101.7	37.15	(35.51, 38.86)	37.49	(35.76, 39.29)	39.53	(38.23, 40.87)
Slope, *p* trend[d]		0.001	0.9	0.01	0.3	0.042	<0.001
Free estradiol (pg/ml)							
Q1	≤84.9	0.87	(0.82, 0.92)	0.86	(0.82, 0.90)	0.83	(0.78, 0.87)
Q2	85.0–92.8	0.87	(0.82, 0.92)	0.87	(0.83, 0.92)	0.86	(0.82, 0.91)
Q3	92.9–101.7	0.91	(0.84, 0.98)	0.92	(0.86, 0.98)	0.92	(0.87, 0.98)
Q4	>101.7	1.03	(0.99, 1.08)	1.04	(0.99, 1.09)	1.08	(1.04, 1.12)
Slope, *p* trend[d]		0.056	<0.001	0.063	<0.001	0.085	<0.001
Sex hormone binding globulin (nmol/l)							
Q1	≤84.9	45.42	(41.75, 49.42)	44.93	(41.38, 48.78)	38.98	(36.97, 41.10)
Q2	85.0–92.8	36.53	(34.30, 38.89)	36.27	(34.33, 38.32)	34.57	(33.11, 36.09)
Q3	92.9–101.7	31.75	(29.71, 33.94)	32.04	(29.92, 34.32)	32.92	(31.47, 34.44)
Q4	>101.7	26.66	(25.48, 27.88)	26.9	(25.76, 28.08)	31.66	(30.32, 33.05)
Slope, *p* trend[d]		−0.17	<0.001	−0.17	<0.001	−0.07	<0.001
Androstanediol glucuronide (ng/ml)							
Q1	≤84.9	10.52	(9.50, 11.65)	10.49	(9.47, 11.61)		Not applicable
Q2	85.0–92.8	12.17	(11.23, 13.19)	12.22	(11.23, 13.29)		
Q3	92.9–101.7	12.76	(11.86, 13.72)	12.72	(11.69, 13.84)		
Q4	>101.7	12.6	(11.59, 13.71)	12.63	(11.54, 13.82)		
Slope, *p* trend[d]		0.058	0.01	0.06	0.02		

[a] *Model 1* adjusted for age and race/ethnicity

[b] *Model 2* adjusted for age and race/ethnicity, smoking, alcohol consumption, physical activity

[c] *Model 3* same as model 2 plus testosterone, estradiol, and SHBG mutually adjusted and free testosterone and free estradiol mutually adjusted

[d] Per 1 quartile change in waist circumference

Table 4 Sex steroid hormone and SHBG concentrations by quartiles of percent body fat in men, NHANES III, 1988–1991

Percent body fat		Model 1[a]		Model 2[b]		Model 3[c]	
Quartile	Range (%)	Mean	95% CI	Mean	95% CI	Mean	95% CI
Total testosterone (ng/ml)							
Q1	≤21.4	6.06	(5.77, 6.37)	5.95	(5.66, 6.25)	5.49	(5.28, 5.71)
Q2	21.5–24.9	5.63	(5.33, 5.95)	5.62	(5.35, 5.90)	5.54	(5.35, 5.74)
Q3	25.0–28.6	5.12	(4.90, 5.36)	5.15	(4.94, 5.37)	5.34	(5.22, 5.47)
Q4	>28.6	4.42	(4.18, 4.68)	4.5	(4.27, 4.74)	4.75	(4.57, 4.94)
Slope, *p* trend[d]		−0.1	<0.001	−0.09	<0.001	−0.05	<0.001
Free testosterone (ng/ml)							
Q1	≤21.4	0.109	(0.106, 0.113)	0.108	(0.104, 0.112)	0.109	(0.104, 0.114)
Q2	21.5–24.9	0.111	(0.104, 0.118)	0.11	(0.104, 0.117)	0.111	(0.106, 0.115)
Q3	25.0–28.6	0.107	(0.103, 0.111)	0.107	(0.104, 0.111)	0.108	(0.104, 0.111)
Q4	>28.6	0.096	(0.091, 0.101)	0.097	(0.093, 0.102)	0.095	(0.091, 0.100)
Slope, *p* trend[d]		−0.04	0.011	−0.03	0.006	−0.04	0.001
Total estradiol (pg/ml)							
Q1	≤21.4	35.52	(33.82, 37.30)	35.09	(33.61, 36.64)	34.3	(32.46, 36.23)
Q2	21.5–24.9	35.48	(33.06, 38.08)	35.59	(33.69, 37.60)	34.74	(33.28, 36.27)
Q3	25.0–28.6	35.69	(33.52, 38.01)	35.59	(33.82, 37.45)	35.52	(33.95, 37.15)
Q4	>28.6	37	(35.58, 38.48)	37.45	(35.94, 39.02)	39.33	(37.67, 41.06)
Slope, *p* trend[d]		0.013	0.145	0.02	0.03	0.043	0.001
Free estradiol (pg/ml)							
Q1	≤21.4	0.85	(0.81, 0.90)	0.85	(0.81, 0.89)	0.83	(0.79, 0.88)
Q2	21.5–24.9	0.89	(0.83, 0.96)	0.89	(0.84, 0.95)	0.89	(0.84, 0.94)
Q3	25.0–28.6	0.93	(0.87, 1.00)	0.93	(0.88, 0.98)	0.93	(0.89, 0.98)
Q4	>28.6	1	(0.95, 1.05)	1.01	(0.97, 1.05)	1.03	(0.99, 1.09)
Slope, *p* trend[d]		0.051	<0.001	0.057	<0.001	0.071	<0.001
Sex hormone binding globulin (nmol/l)							
Q1	≤21.4	42.73	(39.74, 45.95)	42.18	(39.54, 45.00)	37.83	(36.30, 39.42)
Q2	21.5–24.9	35.87	(34.56, 37.23)	35.84	(34.73, 36.98)	33.89	(32.58, 35.24)
Q3	25.0–28.6	31.63	(29.53, 33.87)	31.91	(29.80, 34.18)	32.46	(30.97, 34.02)
Q4	>28.6	28.99	(27.12, 30.99)	29.14	(27.37, 31.02)	33.75	(32.20, 35.38)
Slope, *p* trend[d]		−0.13	<0.001	−0.12	<0.001	−0.04	0.003
Androstanediol glucuronide (ng/ml)							
Q1	≤21.4	10.6	(9.67, 11.62)	10.56	(9.65, 11.56)		Not applicable
Q2	21.5–24.9	12.52	(11.48, 13.64)	12.57	(11.39, 13.86)		
Q3	25.0–28.6	12.16	(11.37, 13.00)	12.24	(11.43, 13.11)		
Q4	>28.6	12.73	(11.68, 13.88)	12.64	(11.62, 13.75)		
Slope, *p* trend[d]		0.052	0.019	0.052	0.03		

[a] *Model 1* adjusted for age and race/ethnicity

[b] *Model 2* adjusted for age and race/ethnicity, smoking, alcohol consumption, physical activity

[c] *Model 3* same as model 2 plus testosterone, estradiol, and SHBG mutually adjusted and free testosterone and free estradiol mutually adjusted

[d] Per 1 quartile change in percent body fat

estradiol statistically significantly increased with increasing BMI (Table 2) and waist circumference (Table 3). For percent body fat (Table 4), we observed a statistically significant increasing association after adjusting for the modifiable factors (Model 2), and an even stronger increasing association after further adjusting for total testosterone and SHBG (Model 3). In Model 3, the slope of the increase in total estradiol concentration with increasing body fatness was similar for BMI, waist circumference, and percent body fat. Free estradiol concentration increased with increasing extent of body fatness in all three models; the slope of the increase was greatest for Model 3 and was

comparable for BMI (Table 2), waist circumference (Model 3), and percent body fat (Model 4).

SHBG

SHBG concentration decreased with increasing extent of each measure of body fatness in all models (Tables 2, 3, 4). The extent of the decline in concentration was similar for Models 1 and 2, but was attenuated after additional adjustment for total testosterone and total estradiol (Model 3). In Model 3, the extent of the decline was similar for BMI (Table 2) and waist circumference (Table 3), but less steep for percent body fat (Table 4).

Androstanediol glucuronide

For each measure of body fatness, androstanediol glucuronide concentration was lowest in the bottom quartile and equally high in the top three quartiles. The associations were the same for Models 1 and 2. The slope of the increase in concentration was higher and statistically significant for waist circumference (Table 3) and percent body fat (Table 4) only.

For each hormone and SHBG, we examined whether the patterns differed by age; however, the patterns were generally similar across age (data not shown).

When we cross-classified the men by quartiles of any two of the body fatness measures, the geometric mean hormone concentrations for men in the top quartile of two of the measures and for men in the bottom quartile of two of the measures were similar to the geometric mean concentrations when using only one of the measures (data not shown).

Finally, we estimated how much the men's BMIs, waist circumferences, and percent body fat would have to differ to result in a 2% lower geometric mean hormone concentrations (Table 5). Lower total and free testosterone and SHBG concentrations were observed among men with higher body fatness, whereas lower total and free estradiol and androstanediol glucuronide concentrations were observed among men with lower body fatness. Body fatness was most strongly associated with total testosterone and SHBG concentrations such that a higher BMI of less than 1 kg/m^2, a higher waist circumference of approximately 2 cm, or a 1% higher body fat percentage would result in a 2% lower geometric mean concentration of these two analytes.

Table 5 Change in BMI, waist circumference, and percent body fat associated with a 2% decline in the geometric mean concentration of total testosterone, free testosterone, total estradiol, free estradiol, SHBG and androstanediol glucuronide in men, NHANES III, 1988–1991

Change in body fatness[a]			
	BMI (kg/m^2)	Waist circumference (cm)	Percent body fat (%)
Total testosterone	0.8	2.0	1.1
Free testosterone	2.3	5.2	2.7
Total estradiol	−4.3	−14.9	−5.1
Free estradiol	−1.4	−3.7	−1.8
SHBG	0.7	1.6	0.9
Androstanediol glucuronide	−6.7	−7.3	−1.9

[a] Estimates are calculated based on the geometric mean testosterone concentration for a 50-year-old white man who is a non-smoker, in the second quartile of alcohol consumption and physical activity

Discussion

We evaluated the associations between body fatness and circulating concentrations of sex steroid hormones and SHBG in a nationally representative sample of US men 20+ years old. We used three measures of body fatness: BMI, waist circumference, and percent body fat. BMI is the most often used measure of body fatness because it is easy to assess. However, it is an imperfect indicator of extent of fat mass because BMI captures both fat and lean mass. Although BMI tends to stay constant with age, lean body mass declines and fat mass increases [18, 19]. Thus, in younger men, a high BMI is more likely to reflect lean body mass than in older men [19]. Nevertheless, BMI is a strong predictor of cardiovascular, total cancer, and all cause mortality [20]. Waist circumference is considered to be a good indicator of central adiposity and, thus, intra-abdominal fat mass [21], and it has been shown to be a better predictor of cardiovascular disease risk than waist-to-hip ratio in men [22]. Additionally, in the European Prospective Investigation into Cancer and Nutrition (EPIC), it has been shown that both general adiposity and abdominal adiposity were associated with the risk of death, but also supported the use of waist circumference or WHR in addition to BMI in assessing the risk of death [4]. The third indicator that we used in this study was the percentage of total mass that is fat mass. Percent body fat is less frequently used in epidemiologic studies because its measurement requires specialized equipment and is more labor intensive [11]. These three measures reflect different aspects of obesity [11], yet are correlated. Thus, our goal was to examine in detail and compare their associations with hormone levels.

We observed a decrease in *total testosterone* concentration with increasing body fatness, which is consistent with previous studies that used either BMI, waist

circumference, or, in some instances, percent body fat [18, 23–28]. Adjustment for smoking, alcohol drinking, and physical activity, each of which is associated with hormone levels [29], slightly reduced the slope of the decline in concentration for each body fatness measure. Because body fatness and testosterone are both associated with estradiol and SHBG, further adjustment for estradiol and SHBG produced a substantial attenuation of the slope. The slopes of the declines in total testosterone concentration were comparable for each of the three measures of body fatness. *Free testosterone* concentration also declined with all three measures of body fatness and the extent of the decline was similar across all three measures. Only a slight attenuation was observed after further adjustment for smoking, alcohol, and physical activity and further adjustment for total estradiol enhanced the slopes. The slope of the decline after adjustment for age and race/ethnicity was less steep than for total testosterone. However, after multivariable adjustment, including total estradiol, the slopes for free and total testosterone were comparable. These results suggest that investigators should consider taking into account other modifiable risk factors and other hormones when studying the links among body fatness, total testosterone, and chronic diseases.

We observed an increase in *total estradiol* concentration with increasing BMI, waist circumference, and percent body fat primarily after taking into account testosterone and SHBG concentrations, both of which are correlates of estradiol and body fatness. The magnitude of the increase in total estradiol concentration was about the same for each measure of body fatness after multivariable adjustment. Each measure of body fatness was associated with *free estradiol*; the association was enhanced with multivariable adjustment. Although it is well recognized that aromatase in fat tissue catalyzes the conversion of testosterone to estradiol [6], not all studies that have evaluated the extent of body fatness and circulating estradiol have observed a direct association. Vermeulen et al. [30] reported higher estradiol concentrations in obese European men (age 25–62 years) compared with non-obese men, but no association was observed in an Italian study [23]. Muller et al. [31] observed a statistically significant increase in estradiol concentration over quartiles of BMI and waist circumference; this study adjusted for age, smoking, alcohol consumption, physical activity, and the presence of chronic diseases. In a US study, estradiol was not statistically associated with body fat or waist circumference in middle-aged men [32] and no association has been observed in other US studies [25, 33]. A Greek study reported a 77% higher estradiol concentration in elderly men with BMI $\geq$ 30 compared with BMI $<$ 27 kg/m^2, although this difference was of marginal statistical significance [34]. One reason for differences among studies might be that studies did not consistently adjust for confounding factors; our results suggest that taking into account testosterone and SHBG are needed to observe a clear association between body fatness and total estradiol.

An inverse association between obesity and *SHBG* has been reported consistently [23, 24, 26–28, 33, 35]. Some studies have observed associations for BMI and/or waist circumference but not fat mass [18, 25, 32]. We observed inverse associations between each of the three measures of body fatness and SHBG. For each, the association was substantially attenuated after taking into account total testosterone and total estradiol; the association was weakest for percent body fat, although it remained statistically significant. It might be that SHBG is more strongly affected by abdominal than overall obesity.

Androstanediol glucuronide is a dihydrotestosterone metabolite that is considered an indicator of testosterone to dihydrotestosterone conversion by 5-alpha-reductases. We observed a non-linear association between the measures of body fatness and androstanediol glucuronide concentration; levels were equally higher in the top three quartiles of each measure of body fatness. However, the magnitude of the association was weaker and not significant for BMI. This latter result is compatible with previous studies that did not find associations between BMI and blood levels of androstanediol glucuronide [33, 36], although high BMI was related to higher levels of androstanediol glucuronide in male EPIC participants [37]. Based on their own and the observations of another study [38], Suzuki et al. [37] hypothesized that excess body weight might stimulate peripheral androgen metabolism, while lowering overall testosterone concentrations.

To get a better sense of just how large of a change in each measure of body fatness would be necessary to yield the same magnitude of change in any given hormone concentration and to determine which hormones are more greatly affected by body fatness for the purpose of assessing public health impact, we estimated the change in each of the measures of body fatness that would yield a 2% change in the geometric mean hormone concentration. Total testosterone and SHBG concentrations were higher with slightly lower BMI, waist circumference, and percent body fat, implying that even a modest loss of body fat could improve testosterone and SHBG profiles. In contrast, big decreases in body fatness would be needed to reduce total estradiol and androstanediol glucuronide.

The strengths of our study include the standardized measurement of the three measures of body fatness, the measurement of hormone levels with good precision, and the availability of modifiable correlates of hormones in a large, well-characterized group of men who are representative of the general US population. Limitations of our study include the cross-sectional nature of the association

such that we cannot determine whether body fatness affects hormones or vice versa, and the single measurement hormone levels, which may not represent the men's usual levels.

In conclusion, our results suggest that measured BMI, waist circumference, and percent body fat lead to similar inferences about the association between body fatness and hormone levels in men. Investigators should use whichever of the measures of body fatness that is most feasible for their study and is appropriate for the outcome (for example, if diabetes is the outcome, the investigators may be most interested in measuring waist circumference). Additionally, investigators should consider taking into account other modifiable risk factors and other hormones when studying the links among body fatness, total testosterone or SHBG, and chronic diseases.

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