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Relationship of sex steroid hormones with body size and with body composition measured by dual-energy X-ray absorptiometry in US men

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Abstract

Purpose—To evaluate the association of body size – captured via whole body dual-energy x-ray absorptiometry (DXA) and physical measurement – with serum sex steroid hormones and sex hormone binding globulin (SHBG) we utilized cross-sectional data and serum samples from the National Health and Nutrition Examination Survey (NHANES; 1999-2004).

Methods—Testosterone, androstanediol glucuronide (3-alpha-diol-G), estradiol and SHBG were measured via immunoassay in serum samples from a total of 898 adult men (ages 20-90) participating in the morning examination. As part of the NHANES data collection DXA scans and measurements of weight, height and waist circumference were performed by trained staff. Linear regression was used to estimate associations between body size and hormone levels adjusted for potential confounders and NHANES sampling procedures.

Results—Total bone area (cm^2) was inversely associated with total testosterone (ng/mL) [beta=-0.12; p-value<0.01], while bone mineral density (g/cm^2) was inversely associated with SHBG (nmol/L) [beta=-17.16; p-value=0.01]. Increased percent body fat was associated with lower concentrations of total testosterone [beta=-0.16; p-value<0.01] and SHBG [beta=-1.11; p-value<0.01] and higher concentrations of free estradiol (fg/mL) [beta=12.52; p-value<0.01].

Conclusions—Clinical measures of body fat (measured via DXA scan) and anthropometric measures of body fat (BMI and waist circumference) provided similar inferences regarding the association between increased body fat and hormone levels in men. Increased body fat was associated with lower circulating levels of testosterone (total and free) and SHBG and higher circulating levels of free estradiol in men, while decreased bone mineral density was associated with higher circulating levels of SHBG.

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The authors declare that they have no conflict of interest.

Keywords

dual-energy X-ray absorptiometry; DXA; estradiol; testosterone; androstanediol glucuronide; sex hormone binding globulin; National Health and Nutrition Examination Survey; NHANES; men

Overweight and obesity are associated with increased risk of some cancers [1;2]. It is hypothesized that some of this risk may be partially explained by obesity's influence on sex steroid hormone concentrations, in men [3]. Further, the association between body mass index (BMI) and sex steroid hormones has been characterized in a number of studies [4-8]. Bone mineral density, another component of body size, has also been correlated with sex steroid concentrations [9;10]; however, the associations between hormone concentrations and bone mineral content, bone area, as well as the components of body size, including total mass, lean mass, fat mass and adult stature, are not well understood.

Dual-energy x-ray absorptiometry (DXA) has become one of the most widely accepted methods of measuring soft tissue and bone composition. In 1999 whole-body DXA scans were added to the National Health and Nutrition Examination Survey (NHANES). Data on bone mineral content, bone mineral density, bone area, total mass, fat mass, lean mass and percent body fat are provided by the DXA scan. The aim of this study was to determine whether clinical and anthropometric body size measurements were associated with serum hormone (testosterone, estradiol and androstanediol glucuronide (3-alpha-diol-G)) and sex-hormone binding globulin (SHBG) levels within a representative sample of US men aged 20-99 years. SHBG is the major carrier of testosterone and estradiol in circulation and was used to calculate levels of free testosterone and free estradiol; 3-alpha-diol-G, a major metabolite of dihydroxytestosterone, is a correlate of tissue androgen levels [11].

SUBJECTS AND METHODS

Study population

NHANES is a series of studies conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention to assess health in the US population through an interview in the household followed by an examination in a mobile examination center [12]. Beginning in 1999, NHANES became a continuous survey (reported in 2-year data cycles). In order to select a representative sample of the civilian, non-institutionalized US population, participant sampling followed a multi-stage, stratified, probability-cluster design. The survey design oversampled adolescents, older Americans, Mexican Americans and non-Hispanic blacks to ensure adequate sample sizes for sub-analysis. The examination component consisted of medical, dental and physiological measurements administered by medical personnel. Blood samples were also collected during the examination. A limited amount of surplus serum, urine, and plasma samples are available from NHANES participants who consented for their specimens to be used for future studies. Access to these surplus samples are allowed to investigators through a formal research proposal submission and approval process. The study presented here, utilizing surplus sera for hormone measurements, underwent scientific and institutional review and was approved by the National Center for Health Statistics Institutional Review Board.

The current study utilizes survey data and sera from study years 1999-2004. During this time there were 15,184 male participants, and these men were randomly assigned to morning or afternoon examinations. Our analysis focused on men aged 20 years and older participating in the morning examination (n=3,281). We focused on the morning examination to control for possible effects of diurnal variation in sex hormone production. Hormone and SHBG levels were tested using serum that had been previously selected for inclusion into the

morning organochlorine subsample so the data analysis could take advantage of the sampling and weights already developed for this subsample. A total of 1,085 men who attended the morning exam were in this subsample, and 985 had surplus sera available for hormone measurement. We excluded 76 men because they had '0' values for the sampling weights for the morning fast, indicating that their data would not contribute to the analysis, and 11 individuals because their DXA data were highly variable and therefore not reliable. The resulting analytic sample included data from 898 men.

Hormone and SHBG levels

Serum testosterone, estradiol, 3-alpha-diol-G, and SHBG levels were measured at the Children's Hospital, Boston, MA. Testosterone, estradiol, and SHBG concentrations were measured using the Elecsys 2010 system (Roche Diagnostics, Laval, QC, Canada). 3-alpha-diol-G was measured by enzyme immunoassay using the Direct 3-alpha-Diol-G ELISA kit (ALPCO Diagnostics, Salem, NH). Laboratory methods and quality control statistics were reported previously [13;13]. A subset of 21 samples was repeated to assess reproducibility; the coefficients of variation (CVs) were 3.4% for testosterone, 4.7% for SHBG, 5.2% for 3-alpha-diol-G, and 15.8% for estradiol.

Using methods described by Vermeulen et al.[14], free testosterone was calculated from testosterone and SHBG values while free estradiol was calculated from estradiol and SHBG values.

Body size measurements

As part of the mobile examination participants underwent whole body DXA using a Hologic QDR 4500A fanbeam densitometer (Hologic Inc, Bedford MA). The scan for each participant was analyzed by the University of California-San Francisco, Department of Radiology, using standard radiologic techniques and study specific protocols developed for NHANES [15]. Our analyses used the five NHANES DXA multiple imputation data files released by the National Center for Health Statistics for study years 1999-2004. Multiple imputation was conducted, in part, because data were not missing completely at random, the details of which are described elsewhere [16].

Anthropometric body size measurements, standing height, weight and waist circumference, were measured by trained study personnel during the examination. BMI was calculated as the ratio of weight (kg) to height squared (m^2).

Statistical analysis

Pearson correlation coefficients were calculated to evaluate correlation between the different DXA measurements. Multiple linear regression models were used to estimate adjusted change in hormone concentration associated with increases in body size as measured by 8 body size measurements from the DXA scan, as well as height, BMI, and waist circumference based on physical measurement. Individual models were run for each hormone-body size association adjusting for age, race/ethnicity, smoking status, alcohol consumption and physical activity, as these factors may influence both hormone concentrations and body size.

Effect modification by age and race/ethnicity were evaluated and results were reported overall and stratified by age, as race/ethnicity was not a significant effect modifier. We also examined the hormone-body size association while simultaneously adjusting for the other hormones measured (e.g. total testosterone-BMI association adjusted for SHBG, 3-alpha-diol-G and total estradiol), which facilitated the evaluation of the association with one hormone while holding constant the concentrations of the other hormones.

To account for the complex survey design (including oversampling), survey nonresponse, and post-stratification in NHANES and to obtain estimates that are representative of the civilian non-institutionalized U.S. population, all statistical analyses employed appropriate survey sampling weights [12]. DXA analyses further utilized the five imputed datasets [16]. Statistical significance was determined based on a two-sided p-value less than 0.05. Analyses were conducted using SAS callable SUDAAN v10.0 (RTI International, Research Triangle Park, NC) as implemented in SAS v9.2 (SAS, Cary, NC) to account for the multi-stage sampling procedure utilized in designing NHANES.

RESULTS

Selected demographic and biologic characteristics of the study population weighted by the sampling fraction are shown in Table 1. The weighted median age for the study population was 41.8 years. The race/ethnicity distribution of the study population was 71.6% non-Hispanic white, 10.4% non-Hispanic black, 8.1% Mexican American, and 9.9% men of other races/ethnicities. On average the study participants were 176.6 cm tall, had a BMI of 27.9 kg/m^2 and a waist circumference of 99.6 cm. Based on DXA scan measurements the older men had higher total percent body fat than younger men (31.0% vs. 26.4%) and lower total bone mineral content than younger men (2500.5 grams vs. 2756.4 grams). The mean hormone concentrations were 5.1 ng/mL for total testosterone, 105.9 pg/mL for free testosterone, 37.1 nmol/L for SHBG, 8.3 ng/mL for 3-alpha-diol-G, 33.7 pg/mL for total estradiol and 896.9 fg/mL for free estradiol.

The DXA body size measurement correlations are presented in Table 2. Total bone area was positively correlated with total bone mineral content and total bone mineral density (correlation coefficient = 0.89 and 0.64, respectively). As expected, total mass and its components, fat mass and lean mass, were highly correlated (correlation coefficient = 0.91 and 0.92, respectively). Lean mass including bone mineral content and lean mass excluding bone mineral content were perfectly correlated (correlation coefficient=1.00), therefore we only present results for lean mass excluding bone mineral content.

Hormone associations with DXA measurements

Results from the multiple linear regression models evaluating the association between body size measurements and hormone concentrations are presented in Table 3. Total bone area (in cm^2) was inversely associated with total testosterone (ng/mL) [beta (estimated change in testosterone for a 100 cm^2 increase in total bone area) = -0.12; p-value < 0.01] and free testosterone (ng/mL) concentrations [beta = -2.81; p-value < 0.01]. Total bone area was not associated with SHBG, 3-alpha-diol-G, total estradiol or free estradiol. Higher total bone mineral content (grams) was associated with lower concentrations of testosterone [beta (estimated change in testosterone for a 100 gram increase in bone mineral content) = -0.04; p-value < 0.01], free testosterone [beta = -0.83; p-value = 0.02] and SHBG [beta = -0.35; p-value = 0.02]. Total bone mineral density ($grams/cm^2$) was inversely associated with SHBG [beta (estimated change in SHBG for a 1 unit increase in bone mineral density) = -17.16; p-value = 0.01], but not associated with the hormones measured.

Increased total mass in grams (lean mass and fat mass combined) was associated with lower concentrations of total testosterone [beta (estimated change in testosterone in ng/mL for a 1 kilogram increase in total mass) = -0.04; p-value < 0.01], free testosterone [beta = -0.53; p-value < 0.01], and SHBG [beta = -0.29; p-value < 0.01] and higher concentrations of 3-alpha-diol-G [beta = 0.03; p-value = 0.03] and free estradiol [beta = 3.75; p-value = 0.01]. Total mass was not associated with total estradiol concentration. The beta-coefficients and standard errors for hormone concentrations in men and the components of total mass – fat mass and lean mass excluding bone mineral content – were similar to the values presented

for total mass. Percent body fat was associated with lower concentrations of total testosterone [beta (estimated change in testosterone for a 1% increase in percent body fat) = -0.16; p-value < 0.01], free testosterone [beta = -1.96; p-value < 0.01], and SHBG [beta = -1.11; p-value < 0.01] and higher concentrations of free estradiol [beta = 12.52; p-value < 0.01] but not 3-alpha-diol-G or total estradiol.

Hormone associations with anthropometric body size measurements

BMI was inversely associated with total testosterone [beta (estimated change in testosterone for a 1 kg/m^2 increase in BMI) = -0.15; p-value < 0.01], free testosterone [beta = -1.76; p-value < 0.01] and SHBG [beta = -1.20; p-value < 0.01], and positively associated with 3-alpha-diol-G [beta = 0.10; p-value = 0.04] and free estradiol [beta = 13.57; p-value = 0.01] (Table 3). Similarly, waist circumference was inversely associated with total testosterone [beta (estimated change in testosterone for a 1 cm increase in waist circumference) = -0.06; p-value < 0.01], free testosterone [beta = -0.77; p-value < 0.01] and SHBG [beta = -0.48; p-value < 0.01], and positively associated with 3-alpha-diol-G [beta = 0.05; p-value = 0.03] and free estradiol [beta = 5.99; p-value = 0.01]. BMI and waist circumference were not associated with total estradiol. Standing height was not associated with any of the hormone concentrations measured [all p-values > 0.07].

The associations between body size measurements and hormones with mutual adjustment for other hormones measured are presented in Supplemental Table 1. The inverse associations between the body size measurements and testosterone remained after adjusting for SHBG, 3-alpha-diol-G and total estradiol. In the mutually adjusted models, the inverse associations between body size measurements and SHBG were no longer significant, whereas positive associations between body size measurements and total estradiol became significant. The association between percent body fat and total estradiol when adjusting for covariates alone was null; however, when the model was also adjusted for total testosterone, SHBG and 3-alpha-diol-G, percent body fat was positively associated with total estradiol [beta = 0.79; p-value < 0.01]. After mutual adjustment for total testosterone, SHBG and total estradiol the significant associations between body size measurements and 3-alpha-diol-G remained. Further, percent body fat was positively associated with 3-alpha-diol-G [beta = 0.12; p-value = 0.03] in the model that adjusted for covariates as well as the other hormones measured.

Stratification by age group

Associations of body size measurements and hormone concentration among young adult (men aged 20-44 years), middle-aged (men aged 45-69 years) and older men (men aged 70 years or older) are shown in Table 4. The results described in the remainder of this section were all significantly modified by age-group (p-value interactions ≤0.05). The inverse associations of total bone area, total bone content and total bone mineral density with total testosterone were limited to the youngest age group. Similarly, the inverse associations between total bone area, total bone mineral content and free testosterone were limited to the youngest age group. Among the oldest age group, total bone area, total bone mineral content and total bone mineral density were all positively associated with free testosterone with the numerically largest association between total bone mineral density and free testosterone [beta = 59.22; p-value < 0.01]. For SHBG, the inverse associations with total bone area, total bone mineral content and total bone mineral density were limited to the youngest and oldest age groups with the strongest inverse association in the oldest age group [beta = -77.91; p-value < 0.01].

The inverse associations between total mass and total testosterone as well as total mass and free testosterone were apparent for the young and middle-age groups with the strongest

inverse associations in the youngest age group for both comparisons. The positive association between total mass and 3-alpha-diol-G was limited to the oldest age group. The effect modification by age-group was consistent for the other components of mass, fat mass and lean mass excluding bone mineral content. The association between percent body fat and 3-alpha-diol-G was also modified by age; the positive association was limited to the oldest age group [beta = 0.31; p-value < 0.01].

BMI was inversely associated with total testosterone for all age groups; the magnitude of the association was numerically largest in the youngest age group. The same was true for the association between waist circumference and total testosterone. The association between waist circumference and 3-alpha-diol-G was modified by age; the positive association with 3-alpha-diol-G was apparent only in the oldest age group.

DISCUSSION

In this large, representative sample of US men, total bone mineral content was inversely associated with testosterone (free and total) and SHBG but not associated with the other sex hormones measured, while total bone mineral density was only associated with lower SHBG. Higher total mass and lean mass were associated with lower concentrations of testosterone (free and total) and SHBG and higher concentrations of 3-alpha-diol-G and free estradiol, while higher fat mass and percent body fat were associated with lower concentrations of testosterone (free and total) and SHBG and higher concentrations of free estradiol. Higher BMI and waist circumference were both associated with lower concentrations of total testosterone, free testosterone and SHBG and higher concentrations of 3-alpha-diol-G and free estradiol. Standing height was not associated with either hormone or SHBG concentrations.

There is accumulating evidence that bone mineral density is associated with hormones in men. Estradiol has been positively associated with bone mineral density, such that low estradiol correlates with low bone mineral density as well as fracture risk, in the majority of cross sectional [17-22] and longitudinal studies [10;23;24] to date. Free and total estradiol were not associated with bone mineral density in our study, the null association between bone mineral density and total estradiol has been reported in other studies [25-29] and is consistent with an NHANES III study utilizing data from 1988-1991 and DXA scans of the femur [30].

Associations between bone mineral density and testosterone have been less consistent, [10;18;20-23;25;26;29;31] however, some studies support a positive association between bone mineral density and free/bioavailable [32] testosterone [18;23;25;26;31] or total testosterone [18;22;31]. Age significantly modified the association between bone mineral density and testosterone in our study. Specifically, the inverse association between bone mineral density and testosterone was limited to the youngest age group. We also observed a strong positive association between bone mineral density and free testosterone in older men, which is consistent with the literature [18;23;26;31]. The results from our analysis support the findings of other studies that bone mineral density is inversely associated with SHBG [17;18;21;27;29;30].

In a prior publication using NHANES III data from 1988-1994, Rohrmann and colleagues reported inverse associations between percent body fat, measured by bioelectric impedance, and testosterone, free testosterone and SHBG and positive associations between percent body fat and 3-alpha-diol-G and estradiol (total and free) [8]. Our results using data from 1999-2004 and percent body fat as measured from the whole body DXA scan also show inverse associations between percent body fat and testosterone (total and free) and SHBG,

and a positive association with free estradiol. We did not however, replicate the positive association between percent body fat and 3-alpha-diol-G or total estradiol.

BMI and waist circumference have been consistently associated with decreased testosterone, free testosterone and SHBG [4-6;8]. Specifically these associations were reported in a previous study from NHANES III (n=1265) [8], and in large population-based studies from Oxford, England (n=750),[4] the Netherlands (n=400) [5], and Norway(n=1563) [6]. In a study of Greek men (n=112, age 40+) BMI was inversely associated with serum testosterone although the result did not achieve statistical significance [7]. Associations with 3-alpha-diol-G and estradiol (total and free) have either been less consistent across study populations or not reported. While we reported positive associations between BMI, waist circumference and 3-alpha-diol-G, Rohrmann and colleagues reported a null association between BMI and 3-alpha-diol-G and a positive association between waist circumference and 3-alpha-diol-G [8]. Conversely, Allen and colleagues reported a positive association between BMI and 3-alpha-diol-G and a null association between waist circumference and 3-alpha-diol-G [4]. Other studies evaluating body size and hormones did not evaluate 3-alpha-diol-G [5-7]. Overall, associations between adiposity-related measurements using DXA (percent body fat, total mass, fat mass and lean mass excluding bone mineral content) and hormone levels were similar to the associations for anthropometric measures (BMI and waist circumference).

The inverse association between body size and testosterone and direct association between body size and 3-alpha-diol-G are consistent with the observed associations with prostate cancer. Specifically, studies have reported increased risk of high-grade prostate cancer associated with low testosterone levels [33] as well as increased risk of aggressive prostate cancer with obese BMI [34]. Further, 3-alpha-diol-G is used as a surrogate marker of steroid 5-alpha-reductase enzymatic activity, or more generally of intra-prostatic androgencity, and may also be associated with increased prostate cancer risk [35]. It is likely, however, that the association needs to be evaluated separately for low-grade and high-grade tumors, given the different associations with obese BMI. The positive association between body size and free estradiol is consistent with increased risk of obesity with colon cancer. Specifically, it is hypothesized that estrogens are associated with colon cancer through multiple mechanisms, including independent effects on risk as well as by strong modifying effect on the association of BMI and colon cancer [36]. Specifically it is hypothesized that estrogen may act indirectly on colon cancer risk through its regulatory action on insulin and the insulin-like growth factor (IGF) system [37].

This study has several strengths including the large sample size and well-characterized study population with a nationally representative sample. Other strengths include the standardized measures of clinical and anthropometric body size performed by trained study personnel, precise measurements of hormones, and availability of adjustment factors that are modifiable correlates of both hormones and body size. Study limitations include the cross-sectional study design, which does not provide direct evidence of cause and effect, and that hormone measurements were based on single serum sample. Serum samples were, however, selected from fasting samples drawn in the morning, to minimize effects of diurnal variation in total and free testosterone. An additional limitation is the use of total bone mineral density, which does not permit evaluation using cortical or trabecular bone compartments.

Our study provides further support that increased body fat is associated with lower circulating levels of testosterone (total and free) and SHBG and higher circulating levels of free estradiol. Clinical measures of body fat (percent body fat, total mass and fat mass as measured via DXA scan) and anthropometric measures of body fat (BMI and waist circumference) provided similar inferences regarding the association between increased

body fat and hormone levels in men; however, the associations with DXA measurements need to be replicated in other large population-based samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Selected demographic and biologic characteristics of adult men in the NHANES survey, 1999-2004.

			Age at interview					
	All N = 898		**20-44 n = 409**		**45-69 n = 336**		**70 + n = 153**	
	Median (IQR)		**Median (IQR)**		**Median (IQR)**		**Median (IQR)**	
Age (years)	41.8 (30.9-54.3)		31.8 (25.1-37.1)		53.3 (48.4-59.3)		75.2 (71.6-80.5)	
Race/ethnicity	n	%	n	%	n	%	n	%
Non-Hispanic White	470	71.6	178	65.6	186	77.2	106	86.3
Non-Hispanic Black	161	10.4	87	12.3	56	8.4	18	7.1
Mexican American	200	8.1	107	11.0	68	4.9	25	3.1
Other	67	9.9	37	11.2	26	9.4	4	3.6
Cigarette smoking								
Never	385	44.5	223	54.0	112	33.5	50	31.9
Former	275	26.9	57	15.4	127	36.8	91	59.3
Current	238	28.5	129	30.5	97	29.7	12	8.8
Number of alcohol drinks (per week)								
0	262	27.7	82	21.1	114	34.3	66.0	40.7
less than 1	199	22.6	94	23.5	68	20.1	37.0	28.8
1 to less than 7	230	28.8	137	37.0	71	20.6	22.0	13.8
7 +	171	20.8	72	18.4	75	25.0	24.0	16.8
Physical activity (MET-hours per week of moderate to vigorous activity)								
Inactive	363	33.8	155	33.2	122	31.4	86	49.4
< 3 Met-hours/week	306	36.3	134	35.0	126	38.8	46	33.4
3 + Met-hours/week	229	29.9	120	31.8	88	29.9	21	17.2
Body size measurements	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)
From DXA scan								
Total bone area (cm^2)	2278.6	(9.4)	2283.2	(11.8)	2289.5	(13.6)	2192.6	(17.1)
Total bone mineral content (BMC) (g)	2717.8	(21.5)	2756.4	(27.3)	2706.7	(29.6)	2500.5	(37.8)
Total bone mineral density (g/cm^2)	1.2	(0.0)	1.2	(0.0)	1.2	(0.0)	1.1	(0.0)
Fat mass (g)	25384.6	(433.2)	23843.4	(615.8)	27545.2	(644.2)	25566.0	(650.5)

	All N = 898		Age at interview: 20-44 n = 409		45-69 n = 336		70 + n = 153	
	Median (IQR)		Median (IQR)		Median (IQR)		Median (IQR)	
Lean mass excluding BMC (g)	59869.5	(472.5)	60223.6	(614.6)	60743.4	(645.2)	53070.7	(617.1)
Lean mass including BMC (g)	62587.2	(489.2)	62980.0	(635.7)	63450.1	(663.8)	55571.2	(643.7)
Total mass (lean+fat) (g)	87971.8	(869.5)	86823.4	(1158.3)	90995.3	(1251.2)	81137.2	(1098.5)
Total percent body fat (%)	28.0	(0.2)	26.4	(0.4)	29.5	(0.3)	31.0	(0.5)
From physical measurement								
Height (cm)	176.6	(0.3)	176.9	(0.4)	176.9	(0.5)	172.9	(0.7)
Body mass index (BMI) (kg/m^2)	27.9	(0.3)	27.4	(0.3)	28.8	(0.4)	27.0	(0.4)
Waist circumference (cm)	99.6	(0.6)	96.0	(0.9)	104.1	(0.9)	102.5	(1.0)
Sex steroid hormone and SHBG concentrations								
Testosterone (ng/mL)	5.1	(0.1)	5.6	(0.1)	4.6	(0.1)	4.1	(0.1)
Free testosterone (pg/mL)	105.9	(2.2)	126.9	(2.4)	86.3	(2.7)	55.8	(1.7)
SHBG (nmol/L)	37.1	(0.9)	30.4	(1.1)	41.5	(1.5)	62.6	(2.8)
3-alpha-diol-G (ng/mL)	8.3	(0.3)	8.4	(0.2)	8.3	(0.5)	7.1	(0.5)
Estradiol (pg/mL)	33.7	(1.0)	35.1	(1.3)	32.9	(1.2)	27.9	(1.7)
Free estradiol (fg/mL)	896.9	(31.1)	971.5	(39.3)	845.4	(39.1)	629.0	(48.7)

IQR = Interquartile range
DXA = Dual X-ray absorptiometry

Percents, means and standard errors have been weighted for NHANES sampling and represent characteristics in the U.S. general population.

Table 2

Pearson correlation coefficients[a] for the dual-energy X-ray absorptiometry (DXA) measurements, NHANES 1999-2004.

	Total bone area	Total bone mineral content	Total bone mineral density	Fat mass	Lean mass excluding BMC	Lean mass including BMC	Total mass (lean+fat)	Total percent body fat
Total bone area (cm^2)	1.00							
Total bone mineral content (BMC) (g)	0.89	1.00						
Total bone mineral density (g/cm^2)	0.64	0.91	1.00					
Fat mass (g)	0.43	0.29	0.12	1.00				
Lean mass excluding BMC (g)	0.79	0.70	0.49	0.68	1.00			
Lean mass including BMC (g)	0.80	0.72	0.52	0.68	1.00	1.00		
Total mass (lean+fat) (g)	0.68	0.56	0.35	0.91	0.92	0.92	1.00	
Total percent body fat (%)	0.09	-0.04	-0.15	0.87	0.29	0.28	0.62	1.00

[a] all p-values < 0.001

Table 3

Adjusted associations between sex steroid hormones, SHBG and body size measurements in the NHANES survey, 1999-2004.

	Total bone area (cm^2) per 100 cm^2 increase			Total bone mineral content (BMC) (g) per 100 g increase			Total bone mineral density (g/cm^2)			Fat mass (g) per 1 kg increase			Lean mass excluding BMC (g) per 1 kg increase		
	Beta[a]	**SE**	**p**	**Beta[a]**	**SE**	**p**	**Beta[a]**	**SE**	**p**	**Beta[a]**	**SE**	**p**	**Beta[a]**	**SE**	**p**
Testosterone (ng/mL)	-0.12	0.03	< 0.01	-0.04	0.01	< 0.01	-0.97	0.65	0.14	-0.09	0.01	< 0.01	-0.06	0.01	< 0.01
Free testosterone (pg/mL)	-2.81	0.57	< 0.01	-0.83	0.33	0.02	-8.18	15.63	0.60	-1.14	0.16	< 0.01	-0.77	0.13	< 0.01
SHBG (nmol/L)	-0.43	0.35	0.23	-0.35	0.14	0.02	-17.16	6.00	0.01	-0.59	0.06	< 0.01	-0.47	0.06	< 0.01
3-alpha-diol-G (ng/mL)	0.20	0.12	0.11	0.10	0.06	0.09	4.06	2.30	0.09	0.05	0.03	0.06	0.06	0.02	0.03
Estradiol (pg/mL)	0.16	0.27	0.56	0.14	0.13	0.28	7.72	5.46	0.17	0.13	0.08	0.13	0.11	0.08	0.14
Free estradiol (fg/mL)	4.00	8.16	0.63	4.52	3.98	0.26	265.71	171.32	0.13	7.55	2.58	0.01	6.08	2.40	0.02

	Total mass (lean mass + fat mass) (g) per 1 kg increase			Percent body fat (%)			Standing Height (cm) per 10 cm increase			Body mass index (BMI) (kg/m^2)			Waist circumference (cm)		
	Beta[a]	SE	p	Beta[a]	SE	p	Beta[a]	SE	p	Beta[a]	SE	p	Beta[a]	SE	p
Testosterone (ng/mL)	-0.04	<0.01	< 0.01	-0.16	0.01	< 0.01	-0.06	0.13	0.65	-0.15	0.02	< 0.01	-0.06	0.01	< 0.01
Free testosterone (pg/mL)	-0.53	0.08	< 0.01	-1.96	0.29	< 0.01	-4.32	2.44	0.08	-1.76	0.30	< 0.01	-0.77	0.12	< 0.01
SHBG (nmol/L)	-0.29	0.03	< 0.01	-1.11	0.11	< 0.01	1.78	1.09	0.11	-1.20	0.12	< 0.01	-0.48	0.04	< 0.01
3-alpha-diol-G (ng/mL)	0.03	0.01	0.03	0.07	0.04	0.11	0.23	0.23	0.32	0.10	0.05	0.04	0.05	0.02	0.03
Estradiol (pg/mL)	0.07	0.04	0.12	0.17	0.13	0.18	0.76	1.03	0.47	0.22	0.15	0.17	0.10	0.07	0.13
Free estradiol (fg/mL)	3.75	1.34	0.01	12.52	3.75	< 0.01	8.40	28.80	0.77	13.57	4.93	0.01	5.99	2.13	0.01

*adjusted for age, race/ethnicity, smoking status, alcohol consumption and physical activity

[a]adjusted for age, race/ethnicity, smoking status, alcohol consumption and physical activity

Table 4

Adjusted associations sex steroid hormones, SHBG and body size measurements stratified by age groups in the NHANES survey, 1999-2004.

	Total bone area (cm^2) per 100 cm^2 increase				Total bone mineral content (BMC) (g) per 100 g increase				Total bone mineral density (g/cm^2)				Fat mass (g) per 1 kg increase				Lean mass excluding BMC (g) per 1 kg increase			
Age groups	**Beta[a]**	**SE**	**p**	**p-int [b]**	**Beta[a]**	**SE**	**p**	**p-int [b]**	**Beta[a]**	**SE**	**p**	**p-int [b]**	**Beta[a]**	**SE**	**p**	**p-int [b]**	**Beta[a]**	**SE**	**p**	**p-int [b]**
Testosterone (ng/mL)																				
20-44	-0.21	0.05	< 0.01	< 0.01	-0.09	0.02	< 0.01	< 0.01	-2.94	0.98	< 0.01	0.02	-0.10	0.01	< 0.01	0.01	-0.08	0.01	< 0.01	< 0.01
45-69	-0.04	0.05	0.43		0.01	0.03	0.98		0.68	1.22	0.58		-0.06	0.01	< 0.01		-0.04	0.01	< 0.01	
70+	0.06	0.08	0.45		0.03	0.04	0.46		1.11	1.61	0.49		-0.07	0.03	0.01		-0.01	0.02	0.77	
Free testosterone (pg/mL)																				
20-44	-3.93	0.96	< 0.01	< 0.01	-1.57	0.55	0.01	< 0.01	-41.36	24.95	0.11	0.01	-1.40	0.22	< 0.01	0.01	-1.13	0.22	< 0.01	< 0.01
45-69	-2.33	1.07	0.04		-0.33	0.53	0.53		15.42	23.98	0.52		-0.82	0.18	< 0.01		-0.46	0.16	0.01	
70+	2.23	1.00	0.03		1.53	0.52	0.01		59.22	18.49	< 0.01		-0.55	0.31	0.08		0.44	0.25	0.08	
SHBG (nmol/L)																				
20-44	-0.78	0.36	0.04	0.05	-0.47	0.15	< 0.01	0.01	-19.43	6.22	< 0.01	0.01	-0.59	0.07	< 0.01	0.61	-0.49	0.07	< 0.01	0.43
45-69	0.88	0.72	0.23		0.16	0.27	0.56		-3.26	11.95	0.79		-0.48	0.13	< 0.01		-0.34	0.10	< 0.01	
70+	-2.74	1.27	0.04		-1.92	0.57	< 0.01		-77.91	20.86	< 0.01		-0.96	0.37	0.01		-0.97	0.31	< 0.01	
3-alpha-diol-G (ng/mL)																				
20-44	-0.06	0.09	0.48	0.01	-0.01	0.04	0.87	0.06	0.83	1.78	0.64	0.28	0.02	0.02	0.29	0.01	0.02	0.02	0.42	0.05
45-69	0.52	0.26	0.06		0.24	0.14	0.08		7.97	4.82	0.11		0.07	0.05	0.19		0.10	0.05	0.04	
70+	0.58	0.25	0.02		0.23	0.14	0.11		6.55	5.94	0.28		0.23	0.07	< 0.01		0.11	0.06	0.06	
Estradiol (pg/mL)																				
20-44	0.05	0.47	0.92	0.12	0.01	0.23	0.98	0.22	-0.26	8.98	0.98	0.30	0.07	0.13	0.58	0.61	0.04	0.13	0.77	0.41
45-69	-0.27	0.65	0.69		0.10	0.28	0.72		12.14	10.44	0.25		0.16	0.09	0.09		0.15	0.11	0.20	
70+	2.09	0.73	0.01		1.09	0.41	0.01		38.17	15.20	0.02		0.25	0.18	0.16		0.47	0.17	0.01	
Free estradiol (fg/mL)																				
20-44	6.46	14.88	0.67	0.20	3.62	7.52	0.63	0.25	142.35	284.68	0.62	0.36	6.93	4.11	0.10	0.93	4.89	3.95	0.22	0.82
45-69	-15.05	21.13	0.48		-0.24	8.60	0.98		260.13	324.99	0.43		7.20	3.02	0.02		6.14	3.41	0.08	
70+	50.22	18.12	0.01		30.05	10.18	0.01		1124.78	362.52	< 0.01		8.50	4.05	0.04		12.50	4.45	0.01	

Age groups	Total mass (lean mass + fat mass) (g) per 1 kg increase	Percent body fat (%)	Standing Height (cm) per 10 cm increase	Body mass index (BMI) (kg/m^2)	Waist circumference (cm)

	Beta[a]	SE	p	p-int [b]	Beta[a]	SE	p	p-int [b]	Beta[a]	SE	p	p-int [b]	Beta[a]	SE	p	p-int [b]	Beta[a]	SE	p	p-int [b]
Testosterone (ng/mL)																				
20-44	-0.05	0.01	< 0.01	< 0.01	-0.17	0.02	< 0.01	0.34	-0.12	0.17	0.49	0.13	-0.19	0.02	< 0.01	< 0.01	-0.07	0.01	< 0.01	< 0.01
45-69	-0.03	0.01	< 0.01		-0.14	0.02	< 0.01		-0.04	0.17	0.83		-0.10	0.02	< 0.01		-0.05	0.01	< 0.01	
70+	-0.02	0.01	0.10		-0.13	0.04	< 0.01		0.30	0.20	0.15		-0.12	0.05	0.02		-0.05	0.02	0.01	
Free testosterone (pg/mL)																				
20-44	-0.70	0.11	< 0.01	< 0.01	-2.17	0.38	< 0.01	0.16	-2.80	3.37	0.41	< 0.01	-2.53	0.43	< 0.01	< 0.01	-1.02	0.16	< 0.01	< 0.01
45-69	-0.35	0.09	< 0.01		-1.62	0.43	< 0.01		-9.11	3.60	0.02		-0.90	0.28	< 0.01		-0.47	0.16	< 0.01	
70+	-0.02	0.16	0.92		-1.27	0.44	0.01		6.16	2.66	0.03		-0.62	0.59	0.30		-0.20	0.19	0.29	
SHBG (nmol/L)																				
20-44	-0.30	0.04	< 0.01	0.35	-1.04	0.11	< 0.01	0.92	0.33	1.10	0.76	0.04	-1.19	0.13	< 0.01	0.58	-0.44	0.05	< 0.01	0.30
45-69	-0.22	0.06	< 0.01		-1.12	0.25	< 0.01		6.25	2.30	0.01		-1.02	0.27	< 0.01		-0.42	0.08	< 0.01	
70+	-0.59	0.18	< 0.01		-0.99	0.58	0.10		-1.80	2.57	0.49		-2.14	0.55	< 0.01		-0.96	0.19	< 0.01	
3-alpha-diol-G (ng/mL)																				
20-44	0.01	0.01	0.33	0.02	0.05	0.04	0.17	0.02	-0.07	0.26	0.79	0.34	0.05	0.04	0.24	0.07	0.02	0.02	0.11	0.03
45-69	0.05	0.03	0.08		0.06	0.10	0.57		0.79	0.45	0.08		0.14	0.09	0.13		0.08	0.05	0.11	
70+	0.10	0.03	< 0.01		0.31	0.08	< 0.01		0.21	0.57	0.72		0.32	0.13	0.02		0.09	0.04	0.02	
Estradiol (pg/mL)																				
20-44	0.03	0.07	0.66	0.39	0.10	0.18	0.58	0.73	1.72	1.22	0.17	0.29	0.04	0.23	0.87	0.39	0.06	0.10	0.55	0.59
45-69	0.08	0.05	0.12		0.31	0.16	0.06		-2.18	2.43	0.38		0.38	0.17	0.03		0.15	0.07	0.03	
70+	0.23	0.09	0.02		0.07	0.29	0.80		3.72	2.43	0.13		0.47	0.38	0.23		0.12	0.14	0.38	
Free estradiol (fg/mL)																				
20-44	3.27	2.18	0.14	0.82	11.10	5.19	0.04	0.83	46.10	36.64	0.21	0.23	10.58	7.13	0.15	0.71	5.27	3.02	0.09	0.80
45-69	3.60	1.68	0.04		15.30	4.96	< 0.01		-94.61	76.05	0.22		16.54	6.04	0.01		6.55	2.02	< 0.01	
70+	6.57	2.24	0.01		6.38	6.60	0.34		95.76	64.03	0.14		15.36	9.59	0.12		5.36	3.45	0.13	

*adjusted for age, race/ethnicity, smoking status, alcohol consumption and physical activity

†p-int = p-value for likelihood ratio test of interaction.

[a]adjusted for age, race/ethnicity, smoking status, alcohol consumption and physical activity

[b]p-int = p-value for likelihood ratio test of interaction.