Published in final edited form as:

Clin Endocrinol (Oxf). 2009 January ; 70(1): 26–34. doi:10.1111/j.1365-2265.2008.03300.x.

Relationship of sex steroid hormones with bone mineral density (BMD) in a nationally representative sample of men

Channing J. Paller^{*}, Meredith S. Shiels[†], Sabine Rohrmann[‡], Shehzad Basaria^{*}, Nader Rifai[§], William Nelson[†], Elizabeth A. Platz[†], and Adrian Dobs^{*}

^{*}Department of Endocrinology, Johns Hopkins School of Medicine, Baltimore, MD

[†]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[‡]Division of Clinical Epidemiology, German Cancer Research Center, Heidelberg, Germany

[§]Department of Laboratory Medicine, Children's Hospital Boston and Harvard Medical School, Boston, MA

Summary

Objective—Sex steroid hormones influence bone mineral density (BMD) in women, but are less well-studied in men. We evaluated the association of serum total and free sex steroid hormones and SHBG with osteopaenia in a nationally representative sample of men aged 20 – 90 years.

Design—BMD and sex steroid hormones were measured among participants in NHANES III, a cross-sectional study of the US population.

Population—A total of 1185 adult men in morning examination session of Phase I of NHANES III (1988 – 91).

Measurements—Relation of oestradiol (E₂), testosterone, and SHBG concentrations with BMD. Osteopaenia was defined as 1–2.5 SD below the mean for white men aged 20 – 29 years.

Results—Men in the lowest quartile of free E₂ had 70% increased odds (OR = 1.69, 95% CI 0.95 – 2.98) of osteopaenia compared with men in the highest quartile. Men in the lowest quartile of free testosterone had nearly four times the odds of osteopaenia than those in the highest quartile (OR = 3.82, 95% CI 1.87 – 7.78). Lower concentrations of SHBG appeared protective against osteopaenia (*P*-trend = 0.01). Neither total testosterone nor total E₂ was associated with BMD, although men with clinically low E₂ (< 20 ng/l) had lower BMD (0.930 g/cm², 95% CI 0.88 – 0.98) than men with normal-range E₂ (1.024 g/cm², 95% CI 1.01–1.04; *P* = 0.004). Findings for free E₂ were most pronounced among elderly men, while the findings for free testosterone were most pronounced among younger men.

Conclusions—In this nationally representative study, men with lower free E₂, lower free testosterone, and higher SHBG concentrations in circulation were more likely to have low BMD.

Introduction

Osteoporosis, defined as bone mineral density (BMD) at least 2.5 standard deviations (SD) below the mean of a young adult population¹ is a significant public health problem in men² and women.³ In 1994, men accounted for 26% of 281 000 US hospital discharges for hip

fractures in people over 45.⁴ Both declines in BMD and deterioration in trabecular bone architecture are important determinants of fracture risk.⁵

Testosterone declines with age; 35% of men aged 60 – 70 years and 90% of men aged 80 – 90 years have clinically low free testosterone levels.⁶ In previous studies, clinically low testosterone was found to be associated with increased risk of osteoporosis and fracture.^{7,8} Testosterone replacement increases BMD in hypogonadal men⁹ and in men over 65 years of age with low serum total testosterone.¹⁰ However, data on the relationship between testosterone levels and BMD are limited for adult men with normal testosterone levels.⁸

Testosterone is the main precursor of oestradiol (E₂) in men; this conversion is catalyzed by aromatase in adipose tissue.¹¹ In one study, BMD was significantly lower in men with low bioavailable E₂ concentration [< 53 pmol/l (14 ng/l)].¹² Another study found serum E₂ concentration was positively associated, SHBG was inversely associated, and testosterone was not associated with BMD in men over 55 years.¹³ Free E₂ concentrations decline as age increases, caused partly by increasing levels of SHBG.¹⁴

Serum concentrations of total and free E₂, total and free testosterone, and SHBG were of interest in this study given their previously shown associations with BMD.¹³ Estimated free E₂ and free testosterone were investigated because unbound hormones more accurately represent hormone concentrations readily available to tissues.

The Third National Health and Nutrition Examination Study (NHANES III) is a large, nationally representative sample of Americans with detailed information on BMD.^{1,4} We supplemented the NHANES data with newly measured serum sex steroid hormone concentrations to evaluate the hypothesis that in the general male population, low total and free E₂, low total and free testosterone, and high SHBG are associated with increased risk of osteopaenia.

Study population

NHANES III, conducted between 1988 and 1994 by the National Center for Health Statistics, was a cross-sectional study of the US population aged two months or greater. NHANES III used a multistage, stratified, clustered probability sample with oversampling of Mexican Americans, non-Hispanic blacks, and older Americans, to ensure adequate sample sizes were available for subanalysis.

NHANES III had two phases (1988 – 1991 and 1991 – 1994) from which independent, unbiased national estimates of health and nutrition characteristics can be produced. Of the 33 944 subjects, 16 037 females and 14 781 males were assigned randomly to morning and afternoon examinations. We studied morning male participants ($n = 2205$) to reduce the effects of diurnal sex hormone production. The NHANES III repository held serum for 1637 of these men. Participants were excluded if their age was under 20 years, or they had a history of prostate cancer and possibly received hormonal therapy or were wheelchair-bound or paralyzed ($n = 236$). Additionally, men were excluded for missing testosterone, E₂, or SHBG concentrations ($n = 21$) or for missing data on BMD ($n = 112$), or per cent body fat, physical activity, or smoking status ($n = 57$).

Assessment of BMD

Hologic Bone Densitometers (Bedford, MA) were used to perform dual-energy X-ray absorptiometry (DEXA) of the total region of the proximal femur, which combined the femur neck, the trochanter region, the intertrochanter region and Ward's triangle. Osteopaenia was defined based on World Health Organization criteria as 1– 2.5 SD below the mean BMD for healthy 20 – 29 years-old men.¹⁵ Mean BMD (1.041 g/cm²) and its SD

(0.144) were based on values for 20 – 29 years old non-Hispanic white men in a previously published study of BMD in NHANES III.¹ Only 26 subjects were classified as having osteoporosis (BMD 2.5 SD below the mean) and, because a separate statistical analysis with osteoporosis as the outcome was not possible, these men were excluded from the analysis. Results were similar when the 26 men with osteoporosis were included along with the men with osteopaenia.

Sex steroid hormones

Sex steroid hormone concentrations were measured in blood drawn from each participant in the morning sample, after overnight fasting. The blood was centrifuged, aliquotted, and stored at -70°C . Serum concentrations of total E_2 , total testosterone, and SHBG were measured in Dr Nadir Rifai's laboratory at Children's Hospital (Boston, MA) by competitive electrochemiluminescence immunoassays on a 2010 Elecsys autoanalyser (Roche Diagnostics, Indianapolis, IN). Laboratory technicians were blinded to participant characteristics and samples. Total serum concentrations of testosterone and E_2 detected in the adult men in NHANES III were generally within the range of reference values used for adult men (testosterone $1.94 - 8.33 \mu\text{g/l}$, E_2 $50 \mu\text{g/l}$).¹⁶ Free E_2 and free testosterone levels were estimated from serum total E_2 and testosterone, SHBG, and albumin concentrations using mass action equations.^{17,18} Total E_2 and testosterone are the combination of SHBG-bound levels (considered non-bioactive) and circulating bioavailable levels (albumin-bound and free).

Lowest detection limits were: testosterone $0.2 \mu\text{g/l}$, E_2 $5 \mu\text{g/l}$, and SHBG 3 nmol/l . Coefficients of variation (CV) for quality control specimens analysed along with the NHANES III specimens were as follows: testosterone 5.9% and 5.8% at 2.5 and $5.5 \mu\text{g/l}$; E_2 6.5% and 6.7% at 102.7 and $474.1 \mu\text{g/l}$; and SHBG 5.3% and 5.9% at 5.3 and 16.6 nmol/l . In a separate run, we tested quality control samples with a mean E_2 concentration of $39.4 \mu\text{g/l}$, which is in the range of typical male E_2 concentration; the interassay CV% was 2.5%.

Assessment of covariates

Covariates considered in the analysis were those known to influence either hormone concentrations or BMD. Participants in NHANES III provided information on age, race/ethnicity, smoking status, and physical activity. A standardized method was utilized to code and classify each type of physical activity by rate of energy expenditure.^{19,20} Weekly frequencies of moderate and vigorous walking, jogging or running, biking, swimming, aerobics, dancing, calisthenics, gardening and lifting weights was measured. Other physical activities were considered to be at the moderate level if they met age-specific cut-offs of the metabolic equivalent (METS) of the activity when compared to rest (3 METS for age ranging 20 – 39 years; 2.5 METS for age ranging 40 – 64 years; 2.0 METS for age ranging 65 – 79 years and 1.26 METS for ages above 79 years). Moderate physical activities were categorized into the following frequency quartiles: 0 times/week, 0 – 2.3 times/week, 2.3 – 7.7 times/week and > 7.7 times/week. A trained examiner measured height and weight. Bioelectrical impedance was estimated using a Valhalla 1990B Bio-Resistance Body Composition Analyser (Valhalla Scientific, San Diego, CA). Per cent body fat was calculated from bioelectric impedance and weight measurements using previously published formulas.^{21,22}

Statistical analysis

Statistical analyses used SUDAAN (Research Triangle Park, NC) as implemented in SAS v. 9.1 (Cary, NC) software. In each analysis, we applied sampling weights to account for the specific probabilities of selection for the individual domains that were over-sampled, non-response, and differences between the sample and the total US population.²³ Total E_2 , total

testosterone, SHBG, free E₂, and free testosterone were categorized into quartiles based on their distributions among all men included in the analysis.

Select participant characteristics were compared between those with and without osteopaenia. Both crude and age-adjusted means and proportions were calculated. Multivariable logistic regression was used to estimate the odds ratio (OR) of osteopaenia and 95% confidence interval (CI) by quartile of hormone concentrations, with the highest quartile as the reference group. The initial model was adjusted for age and race-ethnicity. A second model also was adjusted for smoking status, frequency of moderate physical activity, and per cent body fat. In a third model, the hormones were mutually adjusted for each other. Quartiles of total E₂, total testosterone and SHBG were simultaneously included in a model. The model examining free testosterone was adjusted for quartiles of total E₂ and the model examining free E₂ was adjusted for quartiles of total testosterone. We noted that adjustment for per cent body fat and mutual adjustment for other sex steroid hormones produced changes in the association between hormone concentrations and osteopaenia, and thus we only presented the fully adjusted estimates. Data on serum 25 hydroxyvitamin D are also available in NHANES III. Because vitamin D is a determinant of BMD, we examined whether its levels influenced the results. We found vitamin D neither confounded nor modified the associations between sex steroid hormones and BMD that we observed. Therefore, the results we present are not adjusted for vitamin D.

Separate subanalyses were stratified by age group (20 – 44, 45 – 69, > 70 years) and obesity, defined by ≥ 25% body fat. A cross-product term was included in an unstratified model to assess the presence of interaction between quartiles of each hormone and age groups, and to assess the presence of interaction between quartiles of each hormone and obesity. The coefficients for the cross-product terms were evaluated by the Wald test. Tests for trend were calculated to evaluate the presence of dose–response relationships between quartiles of hormones and osteopaenia.

The mean BMD of men with normal concentrations of total testosterone, total E₂, SHBG, and free testosterone were compared to the mean BMD of men with clinically low levels of these hormones. Clinically low cut-points were defined as follows: total testosterone < 3.0 µg/l; total E₂ < 20 ng/l; SHBG < 13 nmol/l and free testosterone < 0.05 µg/l.^{24–26} Means were adjusted for age, race/ethnicity, per cent body fat, smoking status, frequency of physical activity and mutually adjusted for other hormones.

Results

Selected demographic and biological characteristics of this study population are shown in Table 1. All characteristics are weighted by the sampling fractions, and both crude and age-adjusted means and proportions are presented with standard errors. After age adjustment, the mean BMD was 1.07 g/cm² in those with normal bone density and 0.84 g/cm² in those with osteopaenia. The mean age was 41.3 years with an age range of 20 – 90 years. Participants with osteopaenia were significantly older than those with normal BMD (48.2 vs. 39.5 years). Those with osteopaenia were more likely to be non-Hispanic white, and less likely to be non-Hispanic black or Mexican-American than those with normal BMD. Men were approximately evenly divided among never, former, and current smokers (35.2%, 30.7%, and 34.2%, respectively). After adjustment for age, men with osteopaenia had a lower mean per cent body fat than those with normal BMD (23.1% vs. 25.2%, respectively) and were less likely to engage in frequent physical activity (26.0% vs. 33.9%, respectively).

Table 2 presents the association of quartiles of total and free E₂, total and free testosterone, and SHBG with osteopaenia after adjustment for age, race/ethnicity, smoking status,

physical activity, per cent body fat, and mutual adjustment for other hormones. Overall, total E₂ and total testosterone were not statistically significantly associated with osteopaenia (Table 2). However, lower free E₂ and free testosterone levels were associated with osteopaenia. Men in the lowest quartile of free E₂ had greater odds (OR = 1.69, 95% CI 0.95 – 2.98, *P*-trend = 0.04) of osteopaenia compared with men in the highest quartile. A significant dose–response relationship was also seen between lower levels of free testosterone and increased odds of osteopaenia (*P*-trend = 0.04). Men in the lowest quartile of free testosterone had nearly four times the odds of osteopaenia than those in the highest quartile (OR = 3.82; 95% CI 1.87 – 7.78). Lower concentrations of SHBG appeared protective against osteopaenia (*P*-trend = 0.01); men in the lowest quartile of SHBG had less than half the odds of osteopaenia when compared to those in the highest quartile (OR = 0.41; 95% CI 0.18 – 0.96).

Although there was no association between total E₂ and BMD overall, men with clinically low total E₂ (< 20 ng/l) had a lower mean BMD (0.930 g/cm², 95% CI 0.877–0.983) than men with normal range total E₂ (1.024 g/cm², 95% CI 1.012–1.036; *P* = 0.004) after adjustment for age, race/ethnicity, per cent body fat, smoking status, physical activity, and mutual adjustment for other hormones (Table 3). There was no difference in mean BMD between men with clinically low and normal range total (< 3.0 µg/l) or free testosterone (< 0.05 µg/l). When the total testosterone cut-off was lowered to < 2.5 µg/l no significant difference was seen; however, those with total testosterone < 2.0 µg/l appeared to have a lower mean BMD (0.973 g/cm²; 95% CI 0.920 – 1.026) than those with total testosterone ≥ 2.0 µg/l (1.023 g/cm²; 95% CI 1.013–1.033; *P* = 0.06). There was no significant difference in BMD among men with clinically low or normal SHBG.

The associations of total and free E₂, total and free testosterone, and SHBG with osteopaenia among young adult (20 – 44 years old), middle aged (45 – 69 years old), and older (70 years old) men are shown in Table 4. Consistent with the overall null findings, no associations were seen between total E₂ or total testosterone and osteopaenia among older, middle-aged or younger men. The association between low free E₂ and osteopaenia was strongest among older men with nearly four times the odds of osteopaenia in the lowest quartile of free E₂ (OR = 3.73; 95% CI 0.94 – 14.81) when compared with the highest quartile (*P*-trend = 0.07); However, in middle-aged men and younger men, there was no association. The association between free testosterone and osteopaenia was limited to the youngest age group, with the highest odds of osteopaenia in the lowest quartile of free testosterone (OR = 4.03; 95% CI 1.21–13.44; *P*-trend = 0.02; *P*-interaction = 0.60). Consistent with the main analysis, the lower odds of osteopaenia observed in the bottom three quartiles of SHBG compared with the highest quartile was seen in each age group.

The associations of the hormones with osteopaenia, stratified by obesity, are shown in Table 5. Low free E₂ (lowest vs. highest quartile: OR = 2.65, 95% CI 0.88 – 8.01; *P*-trend = 0.04) was positively associated with an increased odds of osteopaenia in obese men, but not in non-obese men (*P*-interaction = 0.06). Among men who were not obese, lower concentrations of total testosterone (*P*-trend = 0.004) and free testosterone (*P*-trend < 0.001) were positively associated with greater odds of osteopaenia when compared to men in the highest quartile of each hormone. In contrast, among obese men, there was a suggestion that low total testosterone was protective against osteopaenia (*P*-trend = 0.07), whereas there was no association between low free testosterone and osteopaenia (*P*-trend = 0.58). Lower SHBG levels were strongly associated with decreased odds of osteopaenia in non-obese men (*P*-trend < 0.001); those in the lowest quartile of SHBG were observed to have one-quarter of the odds of osteopaenia when compared to those in the highest quartile (OR = 0.24, 95% CI 0.09 – 0.62). Among obese men, lower SHBG concentrations also appeared to be protective against osteopaenia, though the association was not statistically significant (*P*

interaction = 0.44). No association was seen between total E₂ and osteopaenia among obese or non-obese men.

Discussion

Supporting our hypothesis, we found a statistically significant association between low free E₂ and low free testosterone and osteopaenia, and a protective association between low SHBG and osteopaenia, after adjusting for potential confounding factors. Age appears to be an effect modifier of the hormone and osteopaenia association. In overall analyses, we observed a null association between both total testosterone and total E₂ and osteopaenia.

The association between low free E₂ and increased odds of osteopaenia was strongest among men in the oldest age range (> 70), with no significant associations among the youngest (20 – 44) or middle (45 – 69) age groups. Additionally, the lowest quartile of free E₂ was significantly associated with osteopaenia in obese men but not in men who had lower body fat. However, no association was observed between total E₂ and osteopaenia overall, in age-stratified analysis or in obesity-stratified analyses. The inverse association observed between free testosterone and osteopaenia was strongest among the youngest age group with no significant differences observed among men in the older age groups. The data suggest that low free testosterone may be associated with greater odds of osteopaenia among both obese and non-obese men, though there is evidence for a dose–response relationship only among men who are not obese. Though no association was seen overall and when stratified by age group, non-obese, but not obese, men with low total testosterone were observed to have elevated odds of osteopaenia. There was also a strong statistically significant inverse association between low SHBG and osteopaenia that was consistent across age groups and among both obese and non-obese men.

As BMD decreases with age, most studies have limited their exploration of the relationship between hormones and BMD to older men, but our nationally representative study population expanded the age range to 20 – 90 years. In our study, we did not observe an association between total testosterone or total E₂ and BMD. Our results are consistent with studies reporting no association between total testosterone and BMD.^{27–32} However, two studies found a positive association between total testosterone and BMD^{33,34} and another found a positive association between low total testosterone and bone loss.³⁵ Also consistent with our results, two studies reported no association between total E₂ and BMD.^{31,32} However, the majority of previous studies have observed total E₂ to be positively associated with BMD.^{27,29,30,34,36} This discrepancy may be due to differences in age, as many of the previous studies concentrate on older men.^{29,30,34–36} Additionally, many of the previous studies did not adjust for confounders, such as age, per cent body fat, cigarette smoking, physical activity and other hormones.^{28–30,34,36}

As has been observed in other studies, our study further supports the association between low free testosterone and low BMD and the association between low free E₂ and low BMD. Previous studies in older men^{28,33,34} and across a wide age range^{27,31,32} found a positive association between bioavailable or free testosterone and BMD. In contrast, one study found no association between free testosterone and BMD.²⁹ Additionally, the association observed in our study between free E₂ and osteopaenia is supported by a number of studies that have found free E₂ to be positively associated with BMD.^{27–29,32,34,36} Our findings are also consistent with previous research that found SHBG to be inversely associated with BMD.^{13,32,34,36} Our results support findings in both middle age men³⁷ and older men^{13,34,36} that showed higher serum SHBG concentration to be associated with primary or secondary osteoporosis and positively correlated with low BMD. Additionally, our study was able to

show that the association between higher SHBG and osteopaenia is also present among younger men.

In a review of E₂ and the male skeleton, Khosla *et al.* (2002) concluded that E₂ plays an essential role in bone metabolism in men, possibly accounting for as much as 70% of bone resorption.¹² Smith *et al.* (1994) published a case of a male with an oestrogen receptor mutation, who even in the presence of normal testosterone had marked osteopaenia and elevated markers of bone turnover.³⁸ Furthermore, males with aromatase deficiency and normal levels of testosterone had low BMD and high bone turnover, which resolved with treatment of oestrogen.³⁹

Sex steroid withdrawal results in *in vivo*⁴⁰ and *in vitro* osteoblast apoptosis, and E₂, more than testosterone, modulates osteoblast differentiation *in vivo* via cytokines.⁴¹ A previous study has suggested that a threshold level of bioavailable E₂ (40 pmol/l) is needed for effective skeletal maintenance.¹² While results from our study did not support the importance of a threshold level of free E₂, which is highly correlated with bioavailable E₂ ($r = 0.995$),³⁴ our finding of the significant trend of low free E₂ with osteopaenia in men supports E₂'s importance in skeletal maintenance.

Low free testosterone levels were positively associated with osteopaenia, whereas low SHBG concentrations were inversely associated with osteopaenia. Testosterone levels in men are known to decline with age⁴² and SHBG levels are known to rise in older men and women.⁴³ No association was observed between total testosterone and osteopaenia overall, although in men who were not obese, low total and free testosterone were positively associated with osteopaenia. Deterioration of trabecular architecture has also been seen in hypo-gonadal men.⁴⁴ In our study, men with clinically low total testosterone (< 2.0 µg/l) had a lower mean BMD than men with normal range testosterone. These data together with earlier findings that testosterone replacement for men with testosterone concentrations < 2.0 µg/l resulted in BMD increases greater than five per cent⁹ add to the evidence that testosterone is important for BMD, although less so in obese men, perhaps due to elevated concentrations of E₂ among obese men.

Testosterone, like E₂, appears to stimulate bone turnover, acting directly and indirectly via conversion to E₂, in human osteoblast-like cells to increase androgen receptor expression.⁴⁵ Androgens stimulate bone cell division⁴⁶ and mineralization in human osteoblast-like cells.⁴⁵ Steroid hormones bound to SHBG appear to dissociate too slowly to be considered biologically available for end organs, and thus, high levels of SHBG seem to limit the action of E₂ and testosterone in target tissues⁴³ including bone, which might explain why we observed low levels of free E₂ and free testosterone, but not total E₂ or total testosterone, to be associated with osteopaenia in our study.

One limitation of this study is that the cross-sectional design does not allow for discerning the temporality of the observed associations of free E₂ and free testosterone with osteopaenia. Additionally, the equation that we used to calculate free E₂ has only been verified in postmenopausal women.¹⁸ Finally, the mean age of our study population is notably younger compared to other studies, and the prevalence of osteoporosis is quite low (2.2%). However, the wide age range allows the hormonal association with BMD to be extended to younger age categories. Among those over the age of 50 years, our prevalence estimates of osteopaenia are comparable to other studies of older men. The age-adjusted prevalence of osteopaenia in men over 50 is reported to be 28 – 47%.⁴ In this sample of men in NHANES III, the weighted prevalence of osteopaenia for men over 50 was 33.5%, and was 21.2% overall.

We classified men as having osteopaenia based on bone densitometry, a non-invasive method of screening for fracture development. However, bone architecture assessment by magnetic resonance imaging correlates better with vertebral deformities than does bone densitometry.⁴⁷ Though we cannot comment on the magnitude of the associations of sex steroid hormones with bone architecture, bone density accounts for 40 – 60% of mechanical bone strength, whereas bone architecture measurement accounts for only 25 – 40%.⁴⁸ Thus, the associations we observed for hormones and BMD substantially add to the current base of evidence, and suggest further studies assessing modification by age and obesity. In addition, BMD data available for our population was limited to hip measurements. Given that sex steroids may have more impact on the spine than the hip,⁹ the associations between hormones and spine BMD may be stronger than what we observed for hormones and hip BMD. Although the majority of data discussed above supports the association between hormones and BMD, one study looking at bone loss over 6.5 years in a wide age range of men showed that there was only an association between bone loss and SHBG in men, but no association between bone loss and total E₂ and testosterone or free E₂ and testosterone.³² The variation in results could be attributed to differences in the study design (longitudinal vs. cross-sectional), assay types, site of BMD measurement, and timing of hormonal samples. In our analysis, of the 28 men with clinically low E₂, 11 (39%) had osteopaenia. Clinician awareness of osteopaenia and general decline in BMD is important for treatment and prevention of hip fractures.

To our knowledge this is the first study to examine the association between sex steroid hormones and BMD in a large, nationally representative sample of the US male population. The large sample size and corresponding power of the NHANES III study allows this analysis to detect small differences, even after adjustment for relevant covariates, including measures of obesity. In addition, this study profited from the rigorous and standardized measurement of demographic characteristics, laboratory analyses, and anthropometric measures in the NHANES III study. Furthermore, the persistent relationships between low free E₂, low free testosterone and high SHBG with osteopaenia in men after adjustment for multiple covariates, support prior studies.^{13,28,29,36} These results, now expanding the literature to include a nationally representative sample of adult men, call for large prospective studies looking at BMD and/or fracture risk in association with hormone levels. In particular future studies should explore the question of altering clinical management to screen for osteopaenia in men of all ages who have low hormone levels, but do not meet the clinical definition of hypogonadism.

Acknowledgments

Financial support: this is the fifth study from the Hormone Demonstration Program, which is supported by the Maryland Cigarette Restitution Fund Research Grant Program at the Johns Hopkins Medical Institutions.

References

1. Looker AC, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP, Johnston CC Jr, Lindsay R. Updated data on proximal femur bone mineral levels of US adults. *Osteoporosis International*. 1998; 8:468– 489. [PubMed: 9850356]
2. Orwoll ES, Klein RF. Osteoporosis in men. *Endocrine Review*. 1995; 16:87–116.
3. Centers for Disease Control and Prevention (CDC). Osteoporosis Among Estrogen- Deficient Women- United States. *Journal of the American Medical Association*. 1988–1994; 281:224– 226.
4. Looker AC, Orwoll ES, Johnston CC Jr, Lindsay RL, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP. Prevalence of low femoral bone density in older U.S. adults from NHANES III. *Journal of Bone Mineral Research*. 1997; 12:1761–1768.

5. Legrand E, Chappard D, Pascaretti C, Duquenne M, Krebs S, Rohmer V, Basle MF, Audran M. Trabecular bone microarchitecture, bone mineral density, and vertebral fractures in male osteoporosis. *Journal of Bone Mineral Research*. 2000; 15:13–19.
6. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *Journal of Clinical Endocrinology and Metabolism*. 2001; 86:724–731. [PubMed: 11158037]
7. Jackson JA, Riggs MW, Spiekerman AM. Testosterone deficiency as a risk factor for hip fractures in men: a case-control study. *American Journal of Medical Science*. 1992; 304:4– 8.
8. Kenny AM, Prestwood KM, Marcello KM, Raisz LG. Determinants of bone density in healthy older men with low testosterone levels. *Journal of Gerontology a Biology Science Medical Science*. 2000; 55:M492–M497.
9. Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Holmes JH, Dlewati A, Staley J, Santanna J, Kapoor SC, Attie MF, Haddad JG Jr, Strom BL. Effect of testosterone treatment on bone mineral density in men over 65 years of age. *Journal of Clinical Endocrinology and Metabolism*. 1999; 84:1966–1972. [PubMed: 10372695]
10. Amory JK, Watts NB, Easley KA, Sutton PR, Anawalt BD, Matsumoto AM, Bremner WJ, Tenover JL. Exogenous testosterone or testosterone with finasteride increases bone mineral density in older men with low serum testosterone. *Journal of Clinical Endocrinology and Metabolism*. 2004; 89:503– 510. [PubMed: 14764753]
11. Simpson ER. Role of aromatase in sex steroid action. *Journal of Molecular Endocrinology*. 2000; 25:149–156. [PubMed: 11013343]
12. Khosla S, Melton LJ 3rd, Riggs BL. Clinical review 144: Estrogen and the male skeleton. *Journal of Clinical Endocrinology and Metabolism*. 2002; 87:1443–1450. [PubMed: 11932262]
13. Slemenda CW, Longcope C, Zhou L, Hui SL, Peacock M, Johnston CC. Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens. *Journal of Clinical Investigations*. 1997; 100:1755–1759.
14. Orwoll E, Lambert LC, Marshall LM, Phipps K, Blank J, Barrett-Connor E, Cauley J, Ensrud K, Cummings S. Testosterone and estradiol among older men. *Journal of Clinical Endocrinology and Metabolism*. 2006; 91:1336–1344. [PubMed: 16368750]
15. Genant HK, Cooper C, Poor G, Reid I, Ehrlich G, Kanis J, Nordin BE, Barrett-Connor E, Black D, Bonjour JP, Dawson-Hughes B, Delmas PD, Dequeker J, Ragi Eis S, Gennari C, Johnell O, Johnston CC Jr, Lau EM, Liberman UA, Lindsay R, Martin TJ, Masri B, Mautalen CA, Meunier PJ, Miller PD, Mithal A, Morii H, Papapoulos S, Woolf A, Wei Y, Khaltav N. Interim report and recommendations of the World Health Organization Task-Force for Osteoporosis. *Osteoporosis International*. 1999; 10:259– 264. [PubMed: 10692972]
16. Beers, MH.; Berkow, R. *The Merck Manual of Diagnosis and Therapy*. 17. Medical Services, USMEDSA, USHH; 1999. Internet Edition
17. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *Journal of Clinical Endocrinology and Metabolism*. 1999; 84:3666– 3672. [PubMed: 10523012]
18. Rinaldi S, Geay A, Dechaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, Shore RE, Riboli E, Toniolo P, Kaaks R. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. *Cancer Epidemiology Biomarkers and Prevention*. 2002; 11:1065–1071.
19. Ainsworth BE, Haskell WL, Leon AS, Jacobs DR Jr, Montoye HJ, Sallis JF, Paffenbarger RS Jr. Compendium of physical activities: classification of energy costs of human physical activities. *Medical Science Sports Exercise*. 1993; 25:71– 80.
20. Rohrmann S, Crespo CJ, Weber JR, Smit E, Giovannucci E, Platz EA. Association of cigarette smoking, alcohol consumption and physical activity with lower urinary tract symptoms in older American men: findings from the third National Health and Nutrition Examination Survey. *British Journal of Urology International*. 2005; 96:77– 82.
21. National Center for Health Statistics. Plan and Operation of the Third National Health and Nutrition Examination Survey, 1988–94. Series 1: programs and collection procedures. *Vital and Health Statistics* 1. 1994; 32:1–407.

22. Chumlea WC, Guo SS, Kuczmarski RJ, Flegal KM, Johnson CL, Heymsfield SB, Lukaski HC, Friedl K, Hubbard VS. Body composition estimates from NHANES III bioelectrical impedance data. *International Journal of Obesity Related Metabolic Disorders*. 2002; 26:1596–1609.
23. Plan and operation of the Third National Health and Nutrition Examination Survey, 1988 – 94. Series 1: programs and collection procedures. *Vital Health Statistics*. 1994; 1:1– 407.
24. Smith MR, McGovern FJ, Zietman AL, Fallon MA, Hayden DL, Schoenfeld DA, Kantoff PW, Finkelstein JS. Pamidronate to prevent bone loss during androgen-deprivation therapy for prostate cancer. *New England Journal of Medicine*. 2001; 345:948– 955. [PubMed: 11575286]
25. Bukowski C, Grigg MA, Longcope C. Sex hormone-binding globulin concentration: differences among commercially available methods. *Clinical Chemistry*. 2000; 46:1415–1416. [PubMed: 10973877]
26. Greenspan, FBJ. *Basic and Clinical Endocrinology*. 4. Vol. 755. Appleton & Lange; East Norwalk, Connecticut: 1994.
27. Khosla S, Melton LJ 3rd, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL. Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. *Journal of Clinical Endocrinology and Metabolism*. 1998; 83:2266– 2274. [PubMed: 9661593]
28. Khosla S, Melton LJ 3rd, Atkinson EJ, O'Fallon WM. Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men. *Journal of Clinical Endocrinology and Metabolism*. 2001; 86:3555– 3561. [PubMed: 11502778]
29. Gennari L, Merlotti D, Martini G, Gonnelli S, Franci B, Campagna S, Lucani B, Dal Canto N, Valenti R, Gennari C, Nuti R. Longitudinal association between sex hormone levels, bone loss, and bone turnover in elderly men. *Journal of Clinical Endocrinology and Metabolism*. 2003; 88:5327– 5333. [PubMed: 14602769]
30. Amin S, Zhang Y, Sawin CT, Evans SR, Hannan MT, Kiel DP, Wilson PW, Felson DT. Association of hypogonadism and estradiol levels with bone mineral density in elderly men from the Framingham study. *Annals of Internal Medicine*. 2000; 133:951–963.
31. Keles I, Aydin G, Basar MM, Hayran M, Atalar E, Orkun S, Batislam E. Endogenous sex steroids and bone mineral density in healthy men. *Joint Bone Spine*. 2006; 73:80– 85. [PubMed: 16087380]
32. Bjornerem A, Emaus N, Berntsen GK, Joakimsen RM, Fonnebo V, Wilsgaard T, Oian P, Seeman E, Straume B. Circulating sex steroids, sex hormone-binding globulin, and longitudinal changes in forearm bone mineral density in postmenopausal women and men: the Tromso study. *Calcified Tissue International*. 2007; 81:65– 72. [PubMed: 17612780]
33. Mellstrom D, Johnell O, Ljunggren O, Eriksson AL, Lorentzon M, Mallmin H, Holmberg A, Redlund-Johnell I, Orwoll E, Ohlsson C. Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. *Journal of Bone Mineral Research*. 2006; 21:529– 535.
34. van den Beld AW, de Jong FH, Grobbee DE, Pols HA, Lamberts SW. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. *Journal of Clinical Endocrinology and Metabolism*. 2000; 85:3276– 3282. [PubMed: 10999822]
35. Fink HA, Ewing SK, Ensrud KE, Barrett-Connor E, Taylor BC, Cauley JA, Orwoll ES. Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. *Journal of Clinical Endocrinology and Metabolism*. 2006; 91:3908– 3915. [PubMed: 16849417]
36. Goderie-Plomp HW, van der Klift M, de Ronde W, Hofman A, de Jong FH, Pols HA. Endogenous sex hormones, sex hormone-binding globulin, and the risk of incident vertebral fractures in elderly men and women: the Rotterdam Study. *Journal of Clinical Endocrinology and Metabolism*. 2004; 89:3261– 3269. [PubMed: 15240601]
37. Legrand E, Hedde C, Gallois Y, Degasne I, Boux de Casson F, Mathieu E, Basle MF, Chappard D, Audran M. Osteoporosis in men: a potential role for the sex hormone binding globulin. *Bone*. 2001; 29:90– 95. [PubMed: 11472897]

38. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *New England Journal of Medicine*. 1994; 331:1056–1061. [PubMed: 8090165]
39. Bilezikian JP. Osteoporosis in men. *Journal of Clinical Endocrinology and Metabolism*. 1999; 84:3431– 3434. [PubMed: 10522975]
40. Tomkinson A, Reeve J, Shaw RW, Noble BS. The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone. *Journal of Clinical Endocrinology and Metabolism*. 1997; 82:3128– 3135. [PubMed: 9284757]
41. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocrine Review*. 2000; 21:115–137.
42. Ferrini RL, Barrett-Connor E. Sex hormones and age: a cross-sectional study of testosterone and estradiol and their bioavailable fractions in community-dwelling men. *American Journal of Epidemiology*. 1998; 147:750– 754. [PubMed: 9554416]
43. Selby C. Sex hormone binding globulin: origin, function and clinical significance. *Annals of Clinical Biochemistry*. 1990; 27:532– 541. [PubMed: 2080856]
44. Benito M, Gomberg B, Wehrli FW, Weening RH, Zemel B, Wright AC, Song HK, Cucchiara A, Snyder PJ. Deterioration of trabecular architecture in hypogonadal men. *Journal of Clinical Endocrinology and Metabolism*. 2003; 88:1497–1502. [PubMed: 12679429]
45. Takeuchi M, Kakushi H, Tohkin M. Androgens directly stimulate mineralization and increase androgen receptors in human osteoblast-like osteosarcoma cells. *Biochemistry and Biophysics Research Communications*. 1994; 204:905– 911.
46. Kasperk CH, Wergedal JE, Farley JR, Linkhart TA, Turner RT, Baylink DJ. Androgens directly stimulate proliferation of bone cells in vitro. *Endocrinology*. 1989; 124:1576–1578. [PubMed: 2521824]
47. Wehrli FW, Gomberg BR, Saha PK, Song HK, Hwang SN, Snyder PJ. Digital topological analysis of vivo magnetic resonance microimages of trabecular bone reveals structural implications of osteoporosis. *Journal of Bone Mineral Research*. 2001; 16:1520–1531.
48. Ulrich D, van Rietbergen B, Laib A, Ruegsegger P. The ability of three-dimensional structural indices to reflect mechanical aspects of trabecular bone. *Bone*. 1999; 25:55– 60. [PubMed: 10423022]

Table 1

Selected subject characteristics of 1185 men in NHANES III overall, for those with normal bone mineral density and for those with osteopaenia

Characteristic	Crude values			Age adjusted values	
	Total <i>n</i> = 1,211★	Normal bone density <i>n</i> = 927★	Osteopaenia <i>n</i> = 258★	Normal bone density <i>n</i> = 927★	Osteopaenia <i>n</i> = 258★
Age, years $\bar{x}$	41.3 (0.7)	39.5 (0.8)	48.2 (1.5)	—	—
Age categories, % $\bar{x}$					
20–29 years	27.0 (2.3)	29.6 (2.8)	14.4 (4.0)	—	—
30–39 years	25.4 (2.2)	28.0 (3.1)	14.8 (3.7)	—	—
40–49 years	20.7 (1.5)	19.2 (1.9)	25.1 (2.7)	—	—
50–59 years	11.5 (1.1)	10.7 (1.3)	15.5 (2.7)	—	—
60–69 years	9.5 (1.5)	7.7 (1.2)	16.5 (2.6)	—	—
70–79 years	4.6 (0.6)	3.8 (0.7)	9.1 (1.4)	—	—
80–90 years	1.3 (0.2)	0.9 (0.2)	4.5 (0.8)	—	—
Race-ethnicity, % $\bar{x}$					
Non-Hispanic White	78.6 (3.0)	77.6 (3.3)	82.0 (3.8)	78.3 (3.2)	80.9 (4.1)
Non-Hispanic Black	9.1 (1.3)	10.3 (1.5)	4.5 (0.8)	10.2 (1.4)	4.7 (0.9)
Mexican-American	4.9 (0.8)	5.4 (1.0)	3.0 (0.4)	4.9 (0.9)	3.3 (0.5)
Other	7.5 (2.1)	6.7 (2.0)	10.6 (3.4)	6.6 (1.9)	11.1 (3.8)
Bone mineral density, g/cm ² $\bar{x}$ (Proximal femur)	1.02 (0.01)	1.07 (0.01)	0.83 (< 0.01)	1.07 (0.01)	0.84 (0.01)
Weight, lbs $\bar{x}$	178.3 (1.4)	183.0 (1.8)	160.7 (2.3)	183.5 (1.6)	158.8 (2.1)
Height, inches $\bar{x}$	69.4 (0.2)	69.5 (0.2)	68.7 (0.3)	69.5 (0.2)	68.9 (0.3)
Per cent body fat, % $\bar{x}$	24.7 (0.3)	25.0 (0.4)	23.7 (0.5)	25.2 (0.3)	23.1 (0.5)
Smoking, % $\bar{x}$					
Never	35.2 (2.5)	37.0 (2.6)	28.3 (4.2)	36.3 (2.6)	32.0 (5.0)
Former	30.7 (2.9)	29.5 (3.1)	35.2 (3.9)	29.8 (3.0)	27.7 (3.8)
Current	34.2 (2.3)	33.5 (2.5)	36.5 (4.5)	34.0 (2.8)	40.3 (4.9)
Physical activity category, % $\bar{x}$					
None	7.9 (1.3)	6.8 (1.2)	12.1 (2.6)	7.3 (1.2)	10.3 (2.3)
Low	27.1 (2.0)	26.9 (2.1)	28.2 (3.6)	25.9 (2.2)	31.8 (2.3)
Moderate	32.7 (1.6)	32.0 (1.8)	35.3 (3.6)	32.9 (1.6)	32.0 (1.7)

Characteristic	Crude values		Age adjusted values	
	Total <i>n</i> = 1,211★	Normal bone density <i>n</i> = 927★	Osteopaenia <i>n</i> = 258★	Osteopaenia <i>n</i> = 258★
High	32.2 (3.0)	34.3 (3.6)	24.4 (3.9)	26.0 (3.3)

★ Mean or percentage (standard error).

† Indicates a significant unadjusted difference between normal bone mineral density and osteopaenia (*P* 0.05).

‡ Indicates a significant age-adjusted difference between normal bone mineral density and osteopaenia (*P* 0.05).

Table 2

Association of quartiles of testosterone, E₂, SHBG, free testosterone and free E₂ with osteopaenia in NHANES III

	Quartile	Range	OR	95% CI
Total testosterone (µg/l)	1	0.08–4.25	1.33	(0.56–3.18)
	2	4.27–5.42	1.80	(0.97–3.33)
	3	5.43–6.74	1.81	(1.05–3.13)
	4	6.75–13.82	1.0	Ref
				<i>P</i> -trend = 0.47
Total E ₂ (ng/l)	1	5.00–30.26	0.85	(0.40–1.80)
	2	30.27–35.77	1.18	(0.48–2.87)
	3	35.78–43.94	0.45	(0.19–1.08)
	4	43.95–175.8	1.0	Ref
				<i>P</i> -trend = 0.97
SHBG (nmol/l)	1	5.00–25.30	0.41★	(0.18–0.96)
	2	25.31–34.55	0.44★	(0.25–0.79)
	3	34.56–46.67	0.48★	(0.31–0.73)
	4	46.68–185.60	1.0	Ref
				<i>P</i> -trend = 0.01
Free testosterone (µg/l)	1	0.0009–0.084	3.82★	(1.87–7.78)
	2	0.085–0.108	5.12★	(2.23–11.75)
	3	0.109–0.136	2.47★	(1.10–5.55)
	4	0.137–0.317	1.0	Ref
				<i>P</i> -trend = 0.001
Free E ₂ (ng/l)	1	0.08–0.74	1.69	(0.95–2.98)
	2	0.75–0.90	1.77★	(1.02–3.08)
	3	0.91–1.11	1.23	(0.62–2.44)
	4	1.12–2.99	1.0	Ref
				<i>P</i> -trend = 0.04

Adjusted for age, race/ethnicity, smoking status, physical activity, per cent body fat and mutual adjustment for other hormones.

★ Indicates *P* < 0.05.

Table 3

Mean (95% CI) bone mineral density (g/cm²) among 1165 men with clinically normal and clinically low testosterone, E₂, SHBG, and free testosterone in NHANES III

	N	Mean bone mineral density (g/cm ²)★	95% CI	P-value
Total testosterone (µg/l)	124	1.025	(0.982–1.068)	
Clinically low: < 3.0				
Normal: 3.0	1061	1.022	(1.010–1.034)	0.88
Total testosterone (µg/l)	70	0.996	(0.935, 1.057)	
< 2.5				
2.5	1115	1.023	(1.013, 1.033)	0.36
Total testosterone (µg/l)	28	0.973	(0.920, 1.026)	
< 2.0				
2.0	1157	1.023	(1.013, 1.033)	0.06
Total E ₂ (ng/l)	28	0.930	(0.877–0.983)	
Clinically low: < 20				
Normal: 20	1157	1.024	(1.012–1.036)	0.004
SHBG (nmol/l)	19	1.002	(0.955, 1.049)	
Clinically low: < 13				
Normal: 13	1166	1.022	(1.012, 1.032)	0.40
Free testosterone (µg/l)	88	0.995	(0.936–1.054)	
Clinically low: < 0.05				
Normal: 0.05	1097	1.023	(1.013–1.033)	0.32

★ Adjusted for age, race, per cent body fat, smoking status, physical activity, and mutually adjusted for other hormones.

Table 4

Association of quartiles of total testosterone, total E₂, SHBG, free testosterone, and free E₂ with osteopaenia, stratified by categories of age, among 1165 adult men in NHANES III

	Quartile	Age 20–44 years			Age 45–69 years			Age 70 years		
		Range	OR	95% CI	Range	OR	95% CI	Range	OR	95% CI
Total testosterone (µg/l)	1	0.4–4.6	1.63	(0.42–6.29)	0.1–3.6	0.64	(0.16–2.61)	0.08–3.4	0.59	(0.18–1.98)
	2	4.7–5.7	1.26	(0.53–2.98)	3.7–4.6	0.93	(0.34–2.50)	3.5–4.2	0.42	(0.13–1.33)
	3	5.8–7.1	1.80	(0.58–5.62)	4.7–5.9	0.71	(0.28–1.78)	4.3–5.0	2.53	(0.50–12.81)
	4	7.2–13.8	1.0	Ref	6.0–11.0	1.0	Ref	5.1–8.6	1.0	Ref
				<i>P</i> -trend = 0.44			<i>P</i> -trend = 0.54			<i>P</i> -trend = 0.14
				<i>P</i> -interaction = 0.96						
Total E ₂ (ng/l)	1	11.4–30.8	0.72	(0.28–1.84)	5.0–28.8	1.85	(0.78–4.42)	9.6–29.0	2.63	(0.70–9.91)
	2	30.9–36.8	0.69	(0.20–2.30)	28.9–34.0	1.28	(0.46–3.54)	29.1–35.3	2.33	(0.44–12.24)
	3	36.9–44.7	0.27★	(0.08–0.92)	34.1–41.9	3.69★	(1.18–11.57)	35.4–42.3	0.89	(0.24–3.27)
	4	44.8–175.8	1.0	Ref	42.0–107.3	1.0	Ref	42.4–92.1	1.0	Ref
				<i>P</i> -trend = 0.57			<i>P</i> -trend = 0.31			<i>P</i> -trend = 0.15
				<i>P</i> -interaction = 0.53						
SHBG (nmol/l)	1	9.4–23.3	0.25★	(0.07–0.88)	5.0–28.2	0.69	(0.32–1.47)	12.2–42.3	0.33	(0.09–1.26)
	2	23.4–31.2	0.45	(0.16–1.25)	28.3–38.9	0.50	(0.21–1.21)	42.4–54.5	0.20★	(0.05–0.84)
	3	31.3–41.7	0.53	(0.20–1.39)	39.0–53.2	0.37★	(0.14–0.99)	54.6–68.8	0.35	(0.09–1.41)
	4	41.8–163.2	1.0	Ref	53.3–114.7	1.0	Ref	68.9–185.6	1.0	Ref
				<i>P</i> -trend = 0.03			<i>P</i> -trend = 0.13			<i>P</i> -trend = 0.04
				<i>P</i> -interaction = 0.20						
Free testosterone (µg/l)	1	0.002–0.10	4.03★	(1.21–13.44)	0.0009–0.06	2.00	(0.61–6.56)	0.001–0.04	1.32	(0.58–3.03)
	2	0.11–0.12	3.53	(0.85–14.66)	0.07–0.08	1.93	(0.67–5.59)	0.05–0.06	1.53	(0.57–4.11)
	3	0.13–0.14	1.49	(0.44–5.05)	0.09–0.10	3.26★	(1.08–10.47)	0.06–0.08	1.67	(0.41–6.78)
	4	0.15–0.32	1.0	Ref	0.11–0.28	1.0	Ref	0.08–0.14	1.0	Ref
				<i>P</i> -trend = 0.02			<i>P</i> -trend = 0.29			<i>P</i> -trend = 0.59
				<i>P</i> -interaction = 0.60						
Free E ₂ (ng/l)	1	0.29–0.77	1.27	(0.44–3.67)	0.08–0.70	1.75	(0.75–4.08)	0.23–0.64	3.73	(0.94–14.81)

\$watermark-text

\$watermark-text

\$watermark-text

	Age 20-44 years			Age 45-69 years			Age 70 years			
	Quartile	Range	OR	95% CI	Range	OR	95% CI	Range	OR	95% CI
2		0.78-0.96	1.33	(0.44-4.01)	0.71-0.85	1.22	(0.51-2.90)	0.65-0.82	2.58	(0.51-12.97)
3		0.97-1.15	0.69	(0.18-2.62)	0.86-1.02	2.07	(0.71-6.03)	0.83-0.98	3.73	(0.75-10.73)
4		1.16-2.61	1.0	Ref	1.03-2.99	1.0	Ref	0.99-2.86	1.0	Ref
			$P_{\text{trend}} = 0.54$			$P_{\text{trend}} = 0.24$			$P_{\text{trend}} = 0.07$	
			$P_{\text{interaction}} = 0.76$							

Adjusted for age, race/ethnicity, smoking status, physical activity, per cent body fat, and mutually adjusted for hormones.

★ $P < 0.05$.

Table 5

Association of quartiles of total testosterone, total E₂, SHBG, free testosterone and free E₂ with osteopaenia in NHANES III, stratified by obesity

	Quartile	Range	< 25% body fat		25% body fat	
			OR	95% CI	OR	95% CI
Total testosterone (µg/l)	1	0.08–4.25	3.13★	(1.36–7.18)	0.29	(0.06–1.36)
	2	4.27–5.42	3.54★	(1.44–8.70)	0.58	(0.16–2.11)
	3	5.43–6.74	2.88★	(1.44–5.75)	0.88	(0.32–2.40)
	4	6.75–13.82	1.0		1.0	
			<i>P</i> -trend = 0.004		<i>P</i> -trend = 0.07	
Total E ₂ (ng/l)	1	5.00–30.26	0.46	(0.17–1.23)	2.46	(0.54–11.30)
	2	30.27–35.77	0.86	(0.34–2.19)	2.44	(0.58–10.21)
	3	35.78–43.94	0.22★	(0.07–0.71)	1.55	(0.30–7.96)
	4	43.95–175.8	1.0		1.0	
			<i>P</i> -interaction = 0.44		<i>P</i> -trend = 0.15	
SHBG (nmol/l)	1	5.00–25.30	0.24★	(0.10–0.57)	0.84	(0.29–2.42)
	2	25.31–34.55	0.30★	(0.15–0.58)	0.74	(0.36–1.51)
	3	34.56–46.67	0.44★	(0.23–0.83)	0.41★	(0.22–0.76)
	4	46.68–185.60	1.0		1.0	
			<i>P</i> -interaction = 0.09		<i>P</i> -trend = 0.44	
Free testosterone (µg/l)	1	0.0009–0.084	7.26★	(2.36–22.33)	3.88	(0.63–23.82)
	2	0.085–0.108	7.25★	(2.27–23.11)	7.56★	(1.12–51.05)
	3	0.109–0.136	3.33★	(1.08–10.24)	4.14	(0.80–21.33)
	4	0.137–0.317	1.0		1.0	
			<i>P</i> -interaction = 0.49		<i>P</i> -trend = 0.48	
Free E ₂ (ng/l)	1	0.08–0.74	1.24	(0.54–2.80)	2.65	(0.88–8.01)
	2	0.75–0.90	1.49	(0.77–2.89)	2.49	(0.85–7.22)
			<i>P</i> -interaction = 0.80			

	Quartile	Range	< 25% body fat		25% body fat	
			OR	95% CI	OR	95% CI
	3	0.91–1.11	1.21	(0.48–3.01)	1.70	(0.50–5.86)
	4	1.12–2.99	1.0		1.0	
			<i>P</i> -trend = 0.50		<i>P</i> -trend = 0.04	
			<i>P</i> -interaction = 0.06			

Adjusted for age, race/ethnicity, smoking status, physical activity, per cent body fat, and mutual adjustment for other hormones.
★ *P* < 0.05.