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BIOMATERIALS

Relationship of sex steroid hormones with bone mineral density of the lumbar spine in adult men

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Aims

To examine the relationship of sex steroid hormones with osteopenia in a nationally representative sample of men in the USA.

Methods

Data on bone mineral density (BMD), serum sex hormones, dairy consumption, smoking status, and body composition were available for 806 adult male participants of the cross-sectional National Health and Nutrition Examination Survey (NHANES, 1999-2004). We estimated associations between quartiles of total and estimated free oestradiol (E2) and testosterone (T) and osteopenia (defined as 1 to 2.5 SD below the mean BMD for healthy 20- to 29-year-old men) by applying sampling weights and using multivariate-adjusted logistic regression. We then estimated the association between serum hormone concentrations and osteopenia by percentage of body fat, frequency of dairy intake, cigarette smoking status, age, and race/ethnicity.

Results

Men in the lowest quartile of total E2 concentrations (< 21.52 pg/ml) had greater odds of osteopenia compared with men in the highest quartile (odds ratio (OR) 2.29, 95% confidence interval (CI) 1.11 to 4.73; p-trend = 0.030). Total and free T were not associated with osteopenia. Low total E2 concentrations were associated with greater odds of osteopenia among non-daily dairy consumers (p-trend = 0.046), current or former smokers (p-trend = 0.032), and younger men (p-trend = 0.031). No differences were observed by race/ethnicity and obesity.

Conclusion

In this nationally representative study of the USA, men with lower total E2 were more likely to have osteopenia, which was particularly evident among younger men, men with less-than-daily dairy consumption, and current or former smokers.

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Article focus

- We evaluated the association of plasma concentrations of sex steroid hormones with osteopenia in a nationally representative sample of adult men in the USA.

Key messages

- Plasma concentrations of total and free testosterone were not associated with osteopenia in adult men.
- Men with the lowest total oestradiol (E2) concentrations (< 21.52 pg/ml) had twice the odds of osteopenia compared with men with the highest concentrations.
- Low total E2 concentrations were associated with greater odds of osteopenia among non-daily dairy consumers, current or former smokers, and younger men.

Strengths and limitations

- The relatively large sample size allowed for adjusting for many relevant covariates and for examining subgroups of the population.
- The cross-sectional design is a limitation of our study because it allows only a momentary observation of the associations of sex hormones with osteopenia;

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we cannot know whether current hormone levels are those that influenced a man's current bone mineral density (BMD).

- Due to the younger mean age of our study population (42.96 years (95% confidence interval 42.85 to 43.06)), the prevalence of osteoporosis is low (1%), however the wide age range of our study provides additional information on hormonal association with BMD by age.

Introduction

Osteoporosis, which is defined as bone mineral density (BMD) > 2.5 SD below the mean for healthy 20- to 29-year-old men, is a metabolic bone disease that causes a deterioration of bone architecture and results in reduced bone mass and strength, thus elevating fracture risk.[1,2] Its prevalence increases with age and causes high mortality and morbidity among older adults. In 2000, a study estimated nine million osteoporotic fractures among both sexes worldwide, of which 39% occurred in men.[3] In Europe, osteoporotic fractures, after lung cancer, are the main cause of disability-adjusted life-years (DALYs) lost, outranking several other chronic and neoplastic disorders.[3] The lifetime osteoporotic fracture risk for men at age 45 years has been estimated at 25.4%.[4]

In ageing men, an increase in sex hormone binding globulin (SHBG) is observed, which causes a gradual decrease in bioavailable oestradiol (E2). Serum total testosterone (T) also declines with increasing age, which leads to physiological changes such as loss of muscle tone and low BMD.[5–10] In both sexes, it is mainly oestrogen deficiency that causes an imbalance of bone metabolism with an increase of bone resorption, leading to osteopenia (BMD 1 to 2.5 SD below the mean BMD) and osteoporosis.[11]

In this cross-sectional study, we analyzed the associations of serum total and estimated free E2 and T concentrations with osteopenia and BMD across a wide span of ages in men. Additionally, we examined whether age, race/ethnicity, dairy consumption, cigarette smoking, and percentage of body fat modify the association between sex hormone levels and osteopenia. We used existing data from the National Health and Nutrition Examination Survey (NHANES), a nationally representative sample of Americans.[12]

Methods

We included men who participated in NHANES 1999-2004. NHANES is a series of cross-sectional studies conducted by the National Center for Health Statistics (NCHS). Participants represent the noninstitutionalized population of the USA from ages two months and older. The application of a stratified multistage probability design with oversampling of certain population subgroups including Mexican Americans, non-Hispanic blacks, and older adults ensured that adequate sample sizes for subanalysis are available.

Of the 31,126 individuals who were interviewed in NHANES 1999-2004, 15,184 were male. Circulating concentrations of total T, total E2, SHBG, and 3α-androstanediol glucuronide (AAG) for 1,485 males aged 12 years and older were measured in surplus serum, which had been aliquoted and stored at –70°C since the interview. Due to diurnal variation in hormone levels, only blood samples obtained in morning examination sessions were analyzed. Of these 1,485 males we excluded those less than 20 years old (n = 525), missing sampling weights for the morning blood sample (n = 70), and those with missing information on BMD (n = 6). In addition, men were excluded who were previously diagnosed with prostate cancer, because a possible treatment with androgen deprivation therapy affects hormone levels (n = 16).[13] Men who needed special equipment to walk were also excluded, as unloading reduces the mechanical stimulus on bone formation and remodelling, which has a direct effect on BMD (n = 56).[14] After exclusions, 814 men were eligible for inclusion in our analysis. Protocols (#98-12) for NHANES 1999-2004 were approved by the Institutional Review Board of the NCHS, Centers for Disease Control and Prevention (CDC), and all participants provided informed consent.

In NHANES, BMD was measured with dual-energy X-ray absorptiometry (DXA) of the lumbar spine using Hologic Bone Densitometers (Hologic Inc., Marlborough, Massachussetts, USA). T-scores were calculated and osteopenia was defined as 1 to 2.5 SD below the mean BMD for healthy 20- to 29-year-old men according to current National Osteoporosis Foundation (NOF) and World Health Organization (WHO) recommendations.[15,16] Mean BMD (1.064 g/cm^2) and its SD (0.143) were based on values for 20- to 29-year-old non-Hispanic white men in a previously published analysis of BMD in NHANES 1999-2006.[17] A total of eight participants were classified as having osteoporosis (BMD 2.5 SD below the mean). A separate statistical analysis with osteoporosis as the outcome was not possible, therefore these men were excluded from the study. Results were similar when men with osteoporosis were included along with the men with osteopenia (Supplementary Table i).

For NHANES 1999-2004, the participants had blood drawn after an overnight fast. As part of an approved ancillary study, sex steroid hormone concentrations in the selected samples were analyzed in random order at Boston Children's Hospital, Boston, Massachusetts, USA. Plasma concentrations of total E2, total T, and SHBG were measured using a competitive electrochemiluminescence immunoassay on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, Indiana, USA).

All androgens were eliminated via glucuronidation. These metabolites, including AAG, can be measured

Table I. Baseline characteristics of men aged over 20 years with normal bone mineral density of the lumbar spine or osteopenia; National Health and Nutrition Examination Survey 1999-2004

Characteristic*	Total	Normal BMD	Osteopenia†
N (unweighted)	806	623	183
Physical attribute			
Mean age, yrs (95% CI)	42.96 (42.85 to 43.06)	42.92 (42.79 to 43.05)	43.06 (42.83 to 43.28)
Mean BMI, kg/m^2 (95% CI)	27.89 (27.32 to 28.46)	28.41 (27.82 to 28.99)	26.16 (25.20 to 27.11)
Mean body fat, % (95% CI)	27.86 (27.34 to 28.38)	27.85 (27.23 to 28.47)	27.78 (26.77 to 28.79)
Mean waist circumference, cm (95% CI)	99.36 (97.91 to 100.81)	100.43 (98.85 to 102.02)	95.57 (93.27 to 97.88)
Age category, %			
20 to 39 yrs (young adult)	47.2	47.2	47.2
40 to 59 yrs (middle adult)	37.6	37.6	37.5
Over 60 yrs (older adult)	15.3	15.3	15.4
Race and ethnicity, %			
Non-Hispanic White	71.5	71.8	71.1
Non-Hispanic Black	10.5	12.4	3.8
Mexican American	8.1	6.9	12.9
Other Hispanic	4.4	4.9	3.2
Other	5.5	4.0	9.2
Cigarette smoking status, %			
Never	45.5	45.8	46.2
Former	26.3	27.0	23.6
Current	28.2	27.2	30.1
$>$ one alcoholic drink per month, %	57.8	57.2	56.9
$\leqslant$ poverty income ratio, %	15.8	14.6	22.1
Moderate or vigorous physical activity in the last month, %	25.8	25.3	27.7
Dairy once a day or more, %	48.6	50.8	43.9
Milk consumption as a child, %	78.9	80.6	71.9
Milk consumption as a teenager, %	71.1	73.4	63.3
Milk consumption as an adult, %	50.9	52.2	47.4

*Means (95% confidence interval) and proportions are presented taking into account sampling weights and age; n are unweighted.
†Defined as 1 to 2.5 SD below mean bone mineral density.
BMD, bone mineral density; BMI, body mass index; CI, confidence interval.

additionally to total T to reveal the true androgenic activity.[18–20] AAG was measured using enzyme immunoassays (direct androstanediol glucuronide ELISA kit; ALPCO Diagnostics, Salem, New Hampshire, USA). The lowest detection limits of the assays were 5.0 pg/ml for E2, 0.02 ng/ml for T, 0.35 nmol/l for SHBG, and 1.05 nmol/l for AAG. Coefficients of variation (CVs) for embedded quality control samples were: T, 4.8%; AAG, 9.7%; E2, 21.4%; and SHBG, 5.6%. Free T and free E2 were estimated from SHBG, albumin, and total T and E2 concentrations using mass action equations.[21,22]

Information on physical activity, alcohol consumption, cigarette smoking, and dairy consumption was collected during in-person interviews. The height, weight, and waist circumference of the participants were measured by trained personnel. Body fat percentage was estimated with DXA.

Statistical analysis

Statistical analysis was performed using SUDAAN (RTI International, Research Triangle Park, North Carolina, USA) as implemented in SAS v9.1 software (SAS Institute, Cary, North Carolina, USA). In each analysis, sampling weights were applied to account for the specific probabilities of selection for the individual domains that were over-sampled and non-responsive. Total and free E2, total and free T, and AAG were categorized into quartiles based on their distributions among all men included in the analysis. Selected participant characteristics were compared between those with and without osteopenia. Multivariate-adjusted linear regression models were used to compute geometric mean lumbar spine bone density by quartiles, and sex steroid hormone and SHBG concentrations. Model 1 took into account age (continuous) and race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), smoking status (never, current, former), alcohol consumption (more than one alcoholic drink per month, yes or no), moderate or vigorous physical activity in the last month (yes or no), percentage body fat (continuous), current dairy consumption (once a day or more, yes or no), milk consumption as a child (once a day or more, yes or no), milk consumption as a teenager (once a day or more, yes or no), and milk consumption as an adult (once a day or more, yes or no). In model 2, the hormones were mutually adjusted for each other. A test for trend using quartiles of hormones and SHBG as an ordinal variable was performed to assess any statistically significant linear trend.

Multivariate logistic regression was used to estimate the odds ratio (OR) of osteopenia and 95% confidence interval (CI) by quartile of hormone concentrations, with the highest quartile as the reference group, with the adjustments described above.

Subanalyses were stratified by dairy consumption (daily or less than daily), cigarette smoking (ever or never smoker), percentage of body fat ($<$ 25%/$\geqslant$ 25%), age

Table II. Adjusted mean bone mineral density of the lumbar spine (g/cm²) by quartile of serum sex steroid hormones in men aged over 20 years; National Health and Nutrition Examination Survey 1999-2004

Sex steroid hormone	Adjusted mean bone mineral density (95% CI)	
	Model 1*	Model 2†
Total E2 (pg/ml)		
1 (< 21.52)	1.048 (1.009 to 1.087)	1.042 (1.003 to 1.081)
2 (21.52 to < 29.42)	1.041 (1.014 to 1.068)	1.039 (1.012 to 1.066)
3 (29.42 to < 39.29)	1.043 (1.016 to 1.070)	1.044 (1.017 to 1.071)
4 (⩾ 39.29)	1.068 (1.048 to 1.088)	1.072 (1.048 to 1.096)
p-trend‡	0.249	0.124
Free E2 (pg/ml)		
1 (< 0.529)	1.037 (0.996 to 1.078)	1.028 (0.987 to 1.069)
2 (0.529 to < 0.741)	1.040 (1.016 to 1.064)	1.038 (1.014 to 1.062)
3 (0.741 to < 1.003)	1.059 (1.030 to 1.088)	1.060 (1.031 to 1.089)
4 (⩾ 1.003)	1.058 (1.034 to 1.082)	1.064 (1.037 to 1.091)
p-trend‡	0.203	0.103
Total T (ng/ml)		
1 (< 3.67)	1.071 (1.034 to 1.108)	1.077 (1.034 to 1.120)
2 (3.67 to < 4.93)	1.045 (1.021 to 1.069)	1.048 (1.024 to 1.072)
3 (4.93 to < 6.26)	1.048 (1.024 to 1.072)	1.047 (1.023 to 1.071)
4 (⩾ 6.26)	1.042 (1.015 to 1.069)	1.033 (1.004 to 1.062)
p-trend‡	0.300	0.186
Free T (ng/ml)		
1 (< 0.068)	1.100 (1.053 to 1.147)	1.110 (1.059 to 1.161)
2 (0.068 to < 0.095)	1.034 (1.005 to 1.063)	1.038 (1.009 to 1.067)
3 (0.095 to < 0.126)	1.036 (1.018 to 1.054)	1.033 (1.013 to 1.053)
4 (⩾ 0.126)	1.054 (1.027 to 1.081)	1.045 (1.014 to 1.076)
p-trend‡	0.370	0.179
SHBG (nmol/l)		
1 (< 24.05)	1.056 (1.023 to 1.089)	1.048 (1.015 to 1.081)
2 (24.05 to < 33.74)	1.050 (1.023 to 1.077)	1.049 (1.022 to 1.076)
3 (33.74 to < 48.49)	1.045 (1.016 to 1.074)	1.050 (1.019 to 1.081)
4 (⩾ 48.49)	1.048 (1.013 to 1.083)	1.056 (1.017 to 1.095)
p-trend‡	0.717	0.912
AAG (ng/ml)		
1 (< 5.15)	1.038 (1.009 to 1.067)	N/A
2 (5.15 to < 6.89)	1.046 (1.019 to 1.073)	N/A
3 (6.89 to < 9.61)	1.060 (1.031 to 1.089)	N/A
4 (⩾ 9.61)	1.051 (1.029 to 1.073)	N/A
p-trend‡	0.477	N/A

*Model 1 was adjusted for race/ethnicity, age, smoking status, alcohol consumption, moderate or vigorous physical activity in the last month, percentage of body fat, current dairy consumption, milk consumption as a child, milk consumption as a teenager, and milk consumption as an adult.
†Model 2 is as model 1 plus other hormones, excluding 3α-androstanediol glucuronide.
‡A test for trend using quartiles of hormones and sex hormone binding globulin as an ordinal variable was performed to assess any statistically significant linear trend.
AAG, 3α-androstanediol glucuronide; CI, confidence interval; E2, oestradiol; N/A, not applicable; SHBG, sex hormone binding globulin; T, testosterone.

(20 to 39 years, 40 to 59 years, ⩾ 60 years), and race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American). A cross-product term was included in an unstratified model to assess the presence of interaction between quartiles of each hormone and those characteristics. The coefficients for the cross-product terms were evaluated by the Wald test. Statistical significance was set at $p < 0.05$.

Results

Of the men included in the analysis, 22.7% (unweighted n = 183) had osteopenia (Table I).[12] Those with osteopenia were more likely to be non-Hispanic white or Mexican American and less likely to be non-Hispanic black than those with normal BMD. They had a lower mean body mass index (BMI) (26.16 kg/m² (95% CI 25.20 to 27.11) vs 28.41 kg/m² (95% CI 27.82 to 28.99)), lower mean body fat percentage (27.78% (95% CI 26.77 to 28.79) vs 27.85% (95% CI 27.23 to 28.47)), a lower waist circumference (95.57 cm (95% CI 93.27 to 97.88) vs 100.43 cm (95% CI 98.85 to 102.02)), and less often consumed dairy on a daily basis (43.9% vs 50.8%).

Mean BMD did not statistically significantly differ across quartiles of total or free E2 or T, AAG, or SHBG (Table II).[12]

Total E2 was inversely associated with osteopenia, even after adjustment for other hormones. Men in the lowest quartile of total E2 (< 21.52 pg/ml) had greater odds (OR 2.29, 95% CI 1.11 to 4.73; p = 0.030) of osteopenia compared with men in the highest quartile (Table III).[12] The confounder that led to the strongest shift in the association of total E2 with osteopenia was smoking. We did not detect a statistically significant association of free E2, total or free T, SHBG, or AAG with osteopenia.

Since we observed associations between concentrations of total oestradiol with odds of osteopenia, we examined whether results differed by subgroup in the study population (Table IV).[12] Although we did not observe any statistically significant interactions, we noted that the association between E2 and osteopenia was only statistically significant among young men, men who did not consume dairy on a daily basis, and among ever smokers.

Discussion

Numerous studies conducted on osteoporosis only include elderly men. We included men aged 20 to 90 years and, consistent with the majority of other studies, we observed a statistically significantly inverse association between total E2 serum levels and osteopenia after adjusting for potential confounding factors.[23–26] Like several other studies,[23,24,27] we did not observe a statistically significant association between free T and BMD, or SHBG and BMD.[28] Unlike research conducted in the past,[29–31] our results showed no positive association between total T and BMD.

We previously conducted a study on sex steroid hormone levels and BMD in men aged over 20 years in NHANES III (1988-1991).[28] In NHANES III, BMD measured in the proximal femur region was assessed. In the current study, we used BMD of the lumbar spine measured with DXA, as previous research suggested a higher impact of serum sex steroids on the spine than on other regions of the skeleton.[28,32] Other methods[33] to selectively assess the trabecular bone in the vertebrae and measure volumetric bone loss with a higher sensitivity than DXA were not available in NHANES 1999-2004. In contrast to our current results, NHANES III also observed a statistically significant positive association of free E2 and T with

Table III. Odds of osteopenia (lumbar spine) by quartile of serum sex steroid hormone concentration in men aged over 20 years; National Health and Nutrition Examination Survey 1999-2004

Sex steroid hormone	Odds ratio (95% CI)		
	Model 1*	Model 2†	Model 3‡
Total E2 (pg/ml)			
1 (< 21.52)	1.80 (0.88 to 3.66)	2.15 (1.10 to 4.21)	2.29 (1.11 to 4.73)
2 (21.52 to < 29.42)	1.64 (0.88 to 3.02)	1.73 (0.94 to 3.17)	1.81 (0.98 to 3.33)
3 (29.42 to < 39.29)	1.58 (0.78 to 3.21)	1.61 (0.79 to 3.25)	1.61 (0.79 to 3.30)
4 (⩾ 39.29)§	1.00	1.00	1.00
p-trend	0.106	0.037**	0.030**
Free E2 (pg/ml)			
1 (< 0.529)	1.80 (0.87 to 3.69)	1.99 (0.94 to 4.22)	2.05 (0.89 to 4.69)
2 (0.529 to < 0.741)	1.41 (0.73 to 2.75)	1.45 (0.73 to 2.86)	1.39 (0.68 to 2.84)
3 (0.741 to < 1.003)	1.27 (0.69 to 2.35)	1.25 (0.65 to 2.41)	1.21 (0.63 to 2.32)
4 (⩾ 1.003)§	1.00	1.00	1.00
p-trend¶	0.127	0.089	0.116
Total T (ng/ml)			
1 (< 3.67)	0.73 (0.39 to 1.40)	0.90 (0.37 to 2.20)	0.64 (0.22 to 1.87)
2 (3.67 to < 4.93)	1.17 (0.65 to 2.11)	1.23 (0.62 to 2.42)	0.97 (0.45 to 2.10)
3 (4.93 to < 6.26)	0.98 (0.52 to 1.87)	0.99 (0.51 to 1.91)	0.83 (0.40 to 1.72)
4 (⩾ 6.26)§	1.00	1.00	1.00
p-trend¶	0.594	0.941	0.574
Free T (ng/ml)			
1 (< 0.068)	0.86 (0.30 to 2.50)	0.98 (0.31 to 3.08)	0.68 (0.18 to 2.56)
2 (0.068 to < 0.095)	1.89 (0.87 to 4.12)	2.09 (0.93 to 4.70)	1.65 (0.70 to 3.90)
3 (0.095 to < 0.126)	1.74 (0.80 to 3.81)	1.79 (0.81 to 3.96)	1.60 (0.71 to 3.62)
4 (⩾ 0.126)§	1.00	1.00	1.00
p-trend¶	0.835	0.584	0.819
SHBG (nmol/l)			
1 (< 24.05)	0.77 (0.40 to 1.47)	0.93 (0.43 to 2.04)	0.99 (0.43 to 2.28)
2 (24.05 to < 33.74)	1.10 (0.61 to 1.99)	1.18 (0.61 to 2.27)	1.18 (0.56 to 2.46)
3 (33.74 to < 48.49)	0.89 (0.51 to 1.57)	1.01 (0.56 to 1.82)	0.96 (0.51 to 1.81)
4 (⩾ 48.49)§	1.00	1.00	1.00
p-trend¶	0.579	0.943	0.922
AAG (ng/ml)			
1 (< 5.15)	1.63 (0.93 to 2.87)	1.57 (0.86 to 2.88)	N/A
2 (5.15 to < 6.89)	1.10 (0.59 to 2.05)	1.14 (0.61 to 2.13)	N/A
3 (6.89 to < 9.61)	1.07 (0.60 to 1.90)	1.10 (0.61 to 1.98)	N/A
4 (⩾ 9.61)§	1.00	1.00	N/A
p-trend¶	0.133	0.167	N/A

*Adjusted for age and race/ethnicity.
†As with Model 1, plus smoking status, alcohol consumption, moderate or vigorous physical activity in the last month, percentage of body fat, current dairy consumption, milk consumption as a child, milk consumption as a teenager, and milk consumption as an adult.
‡As with Model 2 plus other hormones, excluding 3α-androstanediol glucuronide (AAG).
§Reference point.
¶A test for trend using quartiles of hormones and sex hormone binding globulin as an ordinal variable was performed to assess any statistically significant linear trend.
**Statistically significant.
AAG, 3α-androstanediol glucuronide; CI, confidence interval; E2, oestradiol; N/A, not applicable; SHBG, sex hormone binding globulin; T, testosterone.

BMD.[28] Trabert et al[34] evaluated the association between body composition among the same study population from NHANES 1999-2004, but unlike us they compared total BMD. They observed an inverse association with SHBG, an inverse association with total and free T among the youngest age group, and a positive association with free T among the oldest age group.

Previous research showed that results concerning BMD and sex hormones differ depending on which region of the skeleton was measured. Snyder et al[32] compared the effect of T supplement therapy on BMD of the lumbar spine and the proximal femur and found a statistically significant effect on the lumbar spine only. Kelly et al[35,36] found no association between sex hormone concentration and BMD in either hip or lumbar spine, but they did find a statistically significant positive association between a free T index (ratio of T to SHBG) and BMD at the distal radius. They used single and dual photon absorptiometry to measure BMD, which are less precise than DXA.[35,36] Another alternative for the assessment of bone quality without using DXA are mean cortical bone thickness (CBTavg) and distal femoral cortex index (DFCI).[37] Bilha et al[24] analyzed the correlation between sex hormones and the lumbar spine, hip, forearm, and total body BMD, but only found a statistically significant effect on whole-body BMD.

Our observation that hormone concentrations are only associated with bone health in young men (20 to 39 years old), in non-Hispanic white men, in men with low dairy consumption, or in smokers has, to the best of our

Table IV. Association between serum total oestradiol concentrations and osteopenia (lumbar spine) by percentage of body fat frequency of dairy intake, cigarette-smoking status, age, and race/ethnicity in men aged over 20 years; National Health and Nutrition Examination Survey 1999-2004

Characteristic	Quartile of total E2 concentration (pg/ml)				p-trend*	p-interaction*
	1 (< 21.52)	2 (21.52 to < 29.42)	3 (29.42 to < 39.29)	4 (⩾ 39.29)†		
Total body fat, OR (95% CI)‡						
< 25%	4.43 (0.78 to 25.1)	1.65 (0.37 to 7.37)	1.29 (0.40 to 4.21)	1.00	0.131	N/A
⩾ 25%	1.90 (0.68 to 5.32)	1.99 (0.90 to 4.42)	1.80 (0.69 to 4.70)	1.00	0.149	0.160
Frequency of dairy intake, OR (95% CI)‡						
Daily	1.34 (0.45 to 4.04)	1.33 (0.51 to 3.48)	1.21 (0.34 to 4.33)	1.00	0.581	N/A
< daily or non-consumer	3.22 (1.01 to 10.3)	2.50 (1.03 to 6.03)	1.84 (0.72 to 4.68)	1.00	0.046§	0.579
Cigarette smoking status, OR (95% CI)‡						
Ever	2.45 (0.90 to 6.67)	1.41 (0.61 to 3.23)	1.47 (0.55 to 3.92)	1.00	0.032§	N/A
Never	1.52 (0.41 to 5.61)	2.23 (0.83 to 6.00)	2.01 (0.72 to 5.61)	1.00	0.327	0.339
Age, OR (95% CI)‡						
20 to 39 yrs	5.84 (1.64 to 20.8)	1.65 (0.67 to 4.04)	2.24 (0.75 to 6.71)	1.00	0.031§	N/A
40 to 59 yrs	1.03 (0.22 to 4.72)	2.70 (0.63 to 11.6)	2.34 (0.59 to 9.33)	1.00	0.729	N/A
Over 60 yrs	1.33 (0.35 to 5.08)	2.14 (0.37 to 12.4)	0.29 (0.05 to 1.53)	1.00	0.496	0.684
Race/ethnicity¶, OR (95% CI)‡						
Non-Hispanic White	2.80 (1.09 to 7.17)	1.76 (0.80 to 3.86)	1.98 (0.76 to 5.13)	1.00	0.060	N/A
Non-Hispanic Black	1.00 (0.30 to 3.32)	2.39 (0.56 to 10.3)	0.83 (0.13 to 5.42)	1.00	0.794	N/A
Mexican American	2.09 (0.15 to 28.9)	2.79 (0.44 to 17.6)	3.31 (0.55 to 19.8)	1.00	0.590	N/A¶

*A test for trend using quartiles of hormones and sex hormone binding globulin as an ordinal variable was performed to assess any statistically significant linear trend. Interactions tests were multivariate logistic regression models that included a cross-product term in an unstratified model to assess the presence of interaction between quartiles of each hormone and those characteristics.
†Reference point.
‡If applicable adjusted for age, race/ethnicity, smoking status, alcohol consumption, moderate or vigorous physical activity in the last month, percentage of body fat, current dairy consumption, milk consumption as a child, milk consumption as a teenager, milk consumption as an adult, total testosterone, and sex hormone binding globulin.
§Statistically significant.
¶Results not computable for 'other Hispanic' and 'other race' because of small numbers; no test for interaction computed.
CI, confidence interval; E2, oestradiol; N/A, not applicable; OR, odds ratio.

knowledge, not been described by other studies. Previous observational evidence supports a beneficial effect of dairy intake on bone strength and BMD in older men.[38,39]

To our knowledge, this is one of a few studies that examined the association between sex hormones and osteopenia in young and middle-aged men. With the relatively large sample size and possibility to adjust for many relevant covariates, enabled by the thorough standardized measurement of demographic information, laboratory analyses, and body measurements in NHANES 1999-2004, it was possible to detect even small differences between groups. Unlike other studies, we adjusted for a variety of confounders.[28,40,41]

Limitations of this study include the cross-sectional design. The design allows only a momentary observation of the associations of sex hormones with osteopenia, so we cannot know whether current hormone levels are those that influenced a man's current BMD. Additionally, we did not directly measure free E2 and T and instead estimated them from measured total E2 or T and SHBG. The equation that we used to calculate free E2 has only been verified in postmenopausal women.[22] Due to the younger mean age of our study population (42.96 years (95% CI 42.85 to 43.06)), the prevalence of osteoporosis is low (1%, eight cases), but the wide age range of our study provides additional information on hormonal association with BMD by age. Indeed, a statistically significant association between low total E2 and odds of osteopenia was seen in the youngest but not in the middle and older age groups.

In summary, men with lower total E2 in our study were more likely to have low BMD of the lumbar spine. This was particularly evident among younger men, men who did not consume dairy or consumed dairy less than daily, and ever smokers. These results, which focused on the association between sex hormones and osteopenia in the lumbar spine, should be expanded by studies analyzing the association of hormone levels with other regions of the skeleton and their impact on fracture risk in each region.

Supplementary Material

Table showing results from the National Health and Nutrition Examination Survey 1999-2004, illustrating the effect of sex steroid hormones on adjusted bone mineral density and odds of osteopenia/osteoporosis in men aged over 20 years.

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- K. A. McGlynn: Proofread the manuscript, Contributed input regarding the context.
- S. Rohrmann: Analyzed the data, Proofread the manuscript, Contributed input regarding the context.

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Conflict of interest statement

- E. A. Platz declares grants paid to their institution (Johns Hopkins University) from the American Association of Cancer Research, and the National Institutes of Health, unrelated to this study. E. A. Platz also declares a position on the external advisory board of Kaiser-Permanente Northern California Research Division, unrelated to this study.

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Ethical review statement

- Protocols (#98-12) for NHANES 1999-2004 were approved by the Institutional Review Board of the NCHS, Centers for Disease Control and Prevention (CDC), and all participants provided informed consent.