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Associations between endogenous sex hormone levels and mammographic and bone densities in premenopausal women

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Abstract

Purpose—Mammographic breast and bone mineral densities (BMD) have been associated with luteal phase hormone concentrations in premenopausal women. We assessed the associations of breast and bone densities with follicular phase hormones and sex hormone binding globulin (SHBG) in premenopausal women given that follicular phase hormones have been shown to be positively associated with premenopausal breast cancer risk.

Methods—One hundred and ninety two 40-45 year old women provided a spot urine and/or blood sample during the follicular phase. Hormone and SHBG concentrations and bone density were measured and routine mammograms were accessed and digitized to obtain breast density measures. Regression models were fit to assess the associations between hormones and SHBG and breast and bone densities.

Results—Positive associations were observed between percent breast density and SHBG (p trend = 0.02), as well as estradiol (p trend = 0.08), after controlling for body mass index (BMI), number of pregnancies, and breast feeding history. In addition, a statistically significant inverse association was observed between total testosterone and head BMD (p trend = 0.01), after controlling for BMI.

Conclusions—Associations were observed between breast and bone densities and serum hormone concentrations during the follicular phase of the menstrual cycle.

Keywords

estrogens; androgens; mammographic density; bone mineral density; premenopausal women

Introduction

Circulating sex hormones are implicated in the etiology of certain cancers (e.g., breast, ovarian, and endometrial cancers) and other conditions with high morbidity in women (e.g.,

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endometriosis and polycystic ovarian syndrome) arising in hormone-sensitive tissues (1-4). Lifetime exposure to modestly increased estrogen levels could produce a large cumulative effect on hormone-dependent disease risk. Pike et al. for example, showed that a 20% increase in circulating estrogen concentrations may result in over a 2-fold increase in lifetime breast cancer risk (5).

A single blood measurement may be a useful indicator of long-term hormone levels in premenopausal women, despite fluctuations in plasma hormone concentrations during the menstrual cycle. Intraclass correlation coefficients (ICCs) from a reproducibility study that examined plasma estrogens, androgens, and sex hormone binding globulin (SHBG) by phase showed that a single hormone measure provides a reasonable representation of hormone levels over at least three years, with ICCs ranging from 0.38 (follicular estradiol) to 0.83 (follicular SHBG) (6). However, there is a need for more evidence as to whether a single follicular phase hormone measure can be used to assess long-term premenopausal hormone exposure.

One approach to determine whether lifelong hormone exposure can be characterized by follicular phase hormone concentrations is to assess the relationship between follicular phase hormone concentrations to mammographic breast and bone densities, which are indirect measures of lifelong hormone exposure (7-11). In a well-characterized population of premenopausal women, we assessed the associations of circulating estrogen, androgen, SHBG, and urinary catechol estrogen metabolite concentrations measured during the follicular phase of the menstrual cycle and high and low measures, respectively, of mammographic breast density and BMD.

Materials and methods

Recruitment

As described in detail elsewhere (12), women were recruited from within Group Health (GH), a large integrated health plan in Washington State. We established eligibility criteria to include only premenopausal women who 1) were not currently taking exogenous hormones, 2) had not used hormones at all in the six months prior to the screening mammogram, and 3) had not used hormones for a month or more in the 6-12 months prior to the screening mammogram. A history of hormone use in the study population was defined as the use of oral contraceptives, hormone patches, hormone injections, hormone implants, or intrauterine devices containing progesterone at any time prior to the 6 month-period before the screening mammogram. Women ages 40 – 45 years who had undergone a screening mammogram in the previous 10 months were identified from the GH Breast Cancer Screening Program (13) and recruited based on the Breast Imaging Reporting and Data System (BI-RADS®) density score (14) assigned to their most recent screening mammogram. Our aim was to recruit approximately similar numbers of women with a BI-RADS® density score of 1 or 2 (combined as one group, where 1 = almost entirely fat and 2 = scattered fibroglandular densities), 3 (heterogeneously dense), and 4 (extremely dense). All study procedures were approved by the Institutional Review Boards of the Fred Hutchinson Cancer Research Center (FHCRC) and GH, and all study participants provided written informed consent.

Mammographic breast density data

Because studies have suggested that quantitative (i.e., digitized) measures of breast density create larger gradients in risk of breast cancer than do qualitative measures of breast density, such as BI-RADS (15), each participant's most recent routine GH X-ray screening mammogram prior to their study visit was digitized using a Lumysis 85 scanner (Lumysis, Sunnyvale, CA). Films were read for percent density, dense area size, and total area size by a single reader using Cumulus 108, a computer-assisted mammogram-reading program

developed at the University of Toronto (16). Briefly, the reader uses a sliding scale to outline the breast edge, and the dense area is identified based on pixel brightness. Percent density is the proportion of dense area relative to the total area of the breast. A random selection of 10% of the films was re-read for quality control purposes. The reader was blinded to the original assignment of percent density. A paired t-test comparing the mean percent density of the initial and repeat readings showed no significant difference ($p = 0.81$) and the initial and repeat values were highly correlated (Pearson correlation coefficient: 0.99, $p<0.0001$) with a concordance coefficient of 0.98.

Each woman who undergoes screening mammography at GH completes a risk factor questionnaire on the day of her mammogram. From this questionnaire, we obtained information on the phase of menstrual cycle during which the screening mammogram was taken.

Clinic visits and data collection

Prior to the appointment, self-administered health and demographics questionnaire, physical activity questionnaire, and food frequency questionnaire were mailed to each participant, who was asked to complete them and bring them to the clinic visit. At the clinic visit bone density, weight, height, and waist and hip circumferences were measured, all questionnaires were reviewed for completeness, an early morning blood sample (20 ml) was drawn following an overnight fast, and participants provided a spot urine sample. Blood and urine were transported in coolers with ice packs to the FHCRC Specimen Processing laboratory. Processing took place within two hours of collection. Blood components (i.e., serum, plasma, and buffy coats) and urine aliquots were stored at -70° C. An aliquot of urine was stored for creatinine analysis and the remaining sample was supplemented with 1 part ascorbic acid solution (100 mM ascorbic acid) to 30 parts urine to prevent oxidation of labile estrogen metabolites.

Bone density data

Bone mineral content (BMC) and BMD were measured at the study clinic visit using dual-energy X-ray densitometry [DEXA (Hologic Delphi, Hologic Inc. Bedford, MA)]. Bone density was measured at the anterior-posterior lumbar spine (L1-L4) and proximal femur, including neck and trochanteric regions. In addition, a whole body scan was conducted to provide information on whole body BMC and BMD, and body composition. All measurements were conducted as detailed in the manufacturer's specifications. This included the use of a knee block for spine scans, and medial rotation of the femur with a foot immobilizer for the hip scans. In vitro precision of the machine was monitored throughout the duration of the study in accord with established quality control procedures.

Serum steroid hormone and SHBG concentration analyses

The quantification of serum hormones in this study has been described in detail elsewhere (17). Briefly, estrone (E_1), estradiol (E_2), testosterone (T), androstenedione (A), and dehydroepiandrosterone (DHEA) were quantified by sensitive and specific radioimmunoassays (RIAs) (18,19), and SHBG and DHEA sulfate were quantified by chemiluminescent immunoassay on the Immulite analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Concentrations of free and bioavailable (non-SHBG-bound) E_2 and T were calculated using the measured total E_2 and total T, respectively, and SHBG concentrations and an assumed constant for albumin (20-22). E_1-sulfate (S) was quantified using a highly specific direct RIA (Diagnostic Systems Laboratories, Webster, TX). Two blinded quality control samples were included in each of five batches. The coefficient of variation (CV) % across all QC samples (n=10) ranged from 6.2% for E_2 to 17.2% for E_1S. Mean intra-assay CVs (each CV was based on 2 QC samples per batch) ranged from 3.1% for SHBG to 14.9% for E_1S.

Urinary estrogen metabolite concentration analyses

Measurement of urinary estrogen metabolite concentrations is described in detail elsewhere (17). Briefly, urinary catechol estrogen metabolite concentrations, specifically 2-hydroxy (OH) E_1 and 16α-OH E_1, were measured using a commercially available competitive, solid-phase enzyme-linked immunoassay (ESTRAMET, ImmunaCare, Corp., Bethlehem, PA). To ensure that urine samples were adequately concentrated, urinary creatinine concentrations were measured (12). Based on measured creatinine concentrations, samples were diluted 1:2 or 1:4 prior to testing with manufacturer-supplied diluent. Estrogen metabolite concentrations were determined from a calibration curve derived from six standards supplied with the kit (0.625 – 15.0 ng/ml), and the ratio of 2-OH E_1/16α-OH E_1 was computed. All samples, controls, and standards were assayed in triplicate, and in-house and manufacturer-supplied controls were included in each of the assays performed. Intra-assay and inter-assay CVs for 2-OH E_1 were 4.4% and 8.8%, respectively; for 16α-OH E_1, they were 5.1% and 9.2%, respectively.

Data analysis

Measures of central tendency and categorical distributions were calculated to describe the characteristics of the study population. Initial evaluations of the associations between breast and bone densities and circulating hormone, SHBG, and urinary estrogen metabolite concentrations were performed using correlation analyses. Logistic regression models with robust standard errors were used to assess the associations of percent mammographic breast density and BMD and serum hormone concentrations, SHBG, and estrogen metabolites. We used the change-in-estimate selection method to determine the covariates for our models (23). For the mammographic breast density model, our candidate covariates were age, BMI, number of pregnancies, ethnicity, age at menarche, history of breast feeding, and history of hormone use based on published literature (15). Those variables that, when entered in the model resulted in a 10% or more change in the estimated exposure effect (e.g., serum E_2 concentrations), included BMI, number of pregnancies, and history of breast feeding. For the bone density model, our candidate covariates were age, BMI, age at menarche, and ethnicity (24,25). BMI was the only variable that changed the estimated exposure effect (e.g., serum hormone measures) for BMD by 10% or more. Thus, this variable was included in our final model. BMI was a continuous variable, number of pregnancies was categorized (0, 1, 2+), and history of breast feeding was a dichotomous variable. For total E_2 and total T, SHBG is one of the factors known to determine the amount of free and biologically active hormone and vice versa. As such, these variables were included in the respective models.

Dichotomous outcome variables for breast or bone density were derived by categorizing subjects into the highest quartile (Q4) versus the lower three quartiles (Q1-Q3) combined. For percent breast density, Q4 = 55.0% - 88.9% and Q1-Q3 = 0% -54.9%; for lumbar spine BMD, Q4 = 1.13g/cm^2 – 1.34g/cm^2 and Q1-Q3 = 0.73g/cm^2 – 1.12g/cm^2; for head BMD, Q4 = 2.55g/cm^2 – 3.34g/cm^2 and Q1-Q3 = 1.57g/cm^2 – 2.54g/cm^2; for pelvic BMD, Q4 = 1.24g/cm^2 – 1.51g/cm^2 and Q1-Q3 = 0.83g/cm^2 – 1.23g/cm^2. Serum hormone, catechol estrogen metabolite, and SHBG concentrations were also categorized as quartiles (Q1: ≤25th percentile, Q2: >25th percentile - ≤50th percentile, Q3: >50th percentile - ≤ 75th percentile, Q4: >75th percentile) and odds ratios for the outcome (Q4 vs. Q1-Q3 of breast or bone density) were calculated for each quartile of the exposure with the lowest quartile as the reference. Odds ratios and 95% confidence intervals are presented. We used the Cochrane-Armitage test to assess trends between the ordinal hormone quartile measures and bone and breast density measures. To assess the magnitude of difference in breast and bone densities across the quartiles of hormones, we calculated the median value for these measures by hormone quartile. Data were analyzed using Stata/SE (version 9.0; StataCorp LP, College Station, TX), and a two-sided p-value of ≤ 0.05 was considered statistically significant.

Results

Study population

A total of 1,407 women were identified as potential participants. Of these, 367 (26%) were ineligible, 691 (49%) refused participation, and 146 (10%) were unable to be interviewed or scheduled. A total of 203 women attended a study clinic visit, which was scheduled to occur between days 5 and 9 of the menstrual cycle; 198 (98%) actually attended the clinic visit between days 5 and 9 of their menstrual cycle. One hundred and ninety six women provided a spot urine (n = 195) and/or blood (n = 193) sample. Four participants had E_2 levels > 400 pg/mL. Concentrations this high are considered periovulatory and not typical of concentrations seen between days 5 and 9 of the menstrual cycle; as such, we excluded these four women from our analyses. We also excluded one participant with a mammogram that was too dark to be analyzed for breast density. Therefore, a total of 191 women were included in our breast density analysis and 192 women were included in our bone density analysis.

The mean age of the study participants was 42.4 (SD 1.4) years and the majority had one or more pregnancies, had a history of hormone use, did not currently smoke, were white, and were highly educated (Table 1).

Women in the highest quartile compared to the lower quartiles of breast density had the following characteristics: lower BMI, lower waist-to-hip ratio, lower number of pregnancies, older age at first birth, and absence of a history of breast-feeding (data not shown). Women in the highest quartile versus the lower quartiles of bone density had the following characteristics: higher BMI, higher waist-to-hip ratio, and lower number of pregnancies (data not shown).

Mean (SD) and median (range) values and the correlation matrices of the serum sex hormone concentrations, SHBG, and catechol estrogen metabolites are presented in Table 2. The estrogens were highly correlated, particularly E_2 and free E_2 (r = 0.89). The correlation between T and free T (r = 0.79) was also high. Thus, free E_2 and free T were excluded from further analyses. The estrogens and catechol estrogens were moderately correlated (r > 0.10). The r values between the estrogens and androgens ranged from 0 between E_2 and A to 0.34 between DHEAS and E_1S. Low correlations were observed between mammographic density and lumbar spine BMD (r = -0.11), pelvic BMD (r = - 0.29), and head BMD (r = -0.06) (data not shown).

Associations between endogenous hormone, SHBG, and estrogen metabolite concentrations and percent mammographic breast density

After adjusting for BMI, number of pregnancies, and history of breastfeeding, a borderline significant positive trend was shown between E_2 and percent density [Q2 vs. Q1: 1.36 (95% CI: 0.43, 4.28); Q3 vs. Q1: 1.67 (95% CI: 0.57, 4.90); Q4 vs. Q1: 2.87 (95% CI: 0.98, 8.42); p trend = 0.08; Table 3]. A statistically significant positive association between percent breast density and SHBG was shown [Q2 vs. Q1: OR=6.97 (95% CI: 0.98, 49.3); Q3 vs. Q1: OR=9.84 (95% CI: 1.39, 69.8); Q4 vs. Q1: OR=11.9 (95% CI: 1.66, 85.0); p trend = 0.02; Table 3]. In addition, a borderline significant positive trend was shown between 16α-OH E_1 and breast density (p trend = 0.08), and a significant positive association between percent density and Q3 of 2-OH E_1 compared to Q1 [OR=4.78 (95% CI: 1.36, 16.8); Table 3] was also shown; however, the trend was not significant. A non-significant positive association between androstenedione and breast density, and a non-significant inverse association between DHEA and breast density were also observed (Table 3).

Associations between endogenous hormone, SHBG, and estrogen metabolite concentrations and BMD measures

After adjusting for BMI, no associations between circulating estrogens and any of our three BMD measures (lumbar spine, pelvis, head) were seen (Tables 4-6). However, significant and borderline significant inverse associations were shown between pelvic BMD and 2:16α-OH E_1 and 2-OH E_1 concentrations, respectively (Table 5). A significant inverse association between T and head BMD was observed with women in the highest quartile of head BMD having lower T concentration compared to women in the lower quartiles of head BMD [Q2 vs. Q1: OR=0.72 (95% CI: 0.31, 1.70); Q3 vs. Q1: OR=0.29 (95% CI: 0.10, 0.80); Q4 vs. Q1: OR=0.36 (95% CI: 0.14, 0.94); p-trend = 0.01; Table 6]. Significant inverse associations between androgens and lumbar spine BMD were also seen; however, the trends were not significant (Table 4).

Discussion

In this well-characterized population of healthy premenopausal women, we assessed the associations between breast and bone densities and follicular phase concentrations of estrogens and androgens, SHBG, and urinary catechol estrogen metabolites. To our knowledge, this is the first study that has examined the relationships between concentrations of follicular phase circulating sex hormones and SHBG and mammographic density, and the first study to evaluate the association between catechol estrogen metabolites and mammographic density among premenopausal women.

Our results showing an association of borderline significance between serum E_2 during the follicular phase and percent breast density in premenopausal women are consistent with previous studies that have looked at this association with estrogens during the luteal phase (26,27). The positive association between percent density and E_2 observed in our study is in the hypothesized direction.

We observed a significant positive association between percent breast density and SHBG. Boyd et al reported a similar positive association between mammographic breast density and SHBG in both pre- and postmenopausal women (26); however, SHBG only accounted for 10% of the variance in percent breast density among premenopausal women, which is much lower than 25% of the variance seen in postmenopausal women. This finding, in addition to an inverse association between serum free E_2 concentration and breast density observed by Boyd et al, suggests that estrogenic effects on breast density are not related to the circulating free (i.e., biologically active) form of E_2, and that SHBG may interact directly with binding sites within the breast to promote estrogenic effects (28).

We also observed a significant positive association between percent breast density and 16α-OH E_1. Our findings are similar to studies conducted in postmenopausal women (29). Furthermore, results from Muti et al showed an increase in breast cancer risk with higher concentrations of 16α-OH E_1 in premenopausal women (30) suggesting that 16α-OH E_1 may be involved in the etiology of breast cancer. Our results raise the possibility that the association between 16α-OH E_1 and breast cancer may be mediated, in part, by increasing breast density.

Estrogens are involved in bone growth by inhibiting bone resorption and increasing the production of hormones involved in bone development such as 1,25-dihydroxyvitamin D, growth hormone, and insulin-like growth factor 1 (31). We hypothesized that higher circulating estrogens during the follicular phase would be positively associated with BMD. We did not observe an association between serum estrogen concentrations and BMD. This is consistent with previous findings in premenopausal women during the follicular phase (32,33); however, significant inverse trends were shown between pelvic BMD and urinary 2-OH E_1 and 2:16α-

OH E_1. This is consistent with a previous study in postmenopausal women that showed that women in the lowest quartile of 2-OH E_1:16α-OH E_1 were protected from bone loss over a one year period of follow-up, and higher urinary concentrations of 2-OH E_1 was shown to be associated with lower BMD (34).

We would expect higher T concentrations to be associated with higher BMD due to the aromatization of T into estrogens in fat and other tissues (35), and given the results of a previous study in estrogen-deficient premenopausal women which showed that bone loss (i.e., lower BMD) from the hip was significantly associated with lower androgen concentrations (33). In these healthy, regularly menstruating women, we observed no relationship between T and lumbar or pelvic BMD measures, but a significant inverse association between T and head BMD was observed after adjusting for BMI. Because head BMD is minimally influenced by muscular activity and is relatively free of mechanical stress, it has been hypothesized that head BMD may provide a more accurate assessment of hormonal, genetic, and dietary influences on the mineral status of the skeleton compared to the other more frequently measured BMD sites such as the lumbar spine, hip, and femur (36). In this regard, our findings do not support our hypothesized relationship between androgens and BMD.

There were some strengths and limitations to our study. Premenopausal women tend to have high breast density, so study participants were sampled based on a BI-RADS® classification score, which allowed us to obtain a wide range of breast densities. However, because most women were white and well-educated, reflecting the GH population, and all were insured, our findings may be generalizable only to similar populations of women. Furthermore, bias due to the low participation rate in this study is possible. In the absence of any data on non-participants, bias would only be a concern if characteristics of non-participants that define under- or over-represented segments of the population are also differentially associated with the outcome in those who participate and those who do not. It can be argued that follicular phase hormone measures may not be representative of sex steroid, SHBG, and urinary catechol estrogen metabolite concentrations for premenopausal women throughout the menstrual cycle. Nonetheless, it has been shown that a reasonable characterization of interindividual differences in premenopausal E_2 concentrations can be obtained with single blood samples taken between days 5 and 9 (i.e., early to mid-follicular phase of the menstrual cycle) (37). Further, it has been suggested that among premenopausal women, a single blood measurement can reliably categorize average concentrations of androgens and E_1S over at least a 3-year period (6).

Another potential limitation is that a large number of statistical comparisons were made, suggesting that some of the statistically significant findings may have occurred by chance alone. Finally, the cross-sectional nature of our study cannot establish the temporal sequence of hormone concentrations and breast and bone density measures. Nevertheless, our results provide important baseline information for future research.

In summary, in this population of premenopausal women, higher concentrations of estrogens and SHBG during the follicular phase of the menstrual cycle were associated with higher mammographic breast density. We also observed associations between androgens and head and lumbar spine BMD and urinary estrogen metabolite concentrations and pelvic BMD. Lifetime exposure to increased hormone concentrations is a risk factor for several hormone-dependent diseases. Our results showed some associations between a single hormone measure during the follicular phase of the menstrual cycle and biomarkers of hormone exposure, i.e., breast and bone densities. As such, identification of high-risk women for hormone-dependent diseases for prevention efforts may be possible. Further studies involving a larger sample of more diverse populations are needed to confirm and improve the generalizability of these findings.

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Table 1

Characteristics of study population: Group Health, Seattle, WA – Years of recruitment 2004 - 2005 (N = 192*)

Age, y (mean, SD)	42.4	(1.4)
Age at menarche, y (mean, SD)	12.8	(1.3)
Age at first birth§, y (mean, SD)	29.0	(5.8)
BMI, kg/m^2 (mean, SD)	25.7	(4.6)
Height, m (mean, SD)	1.65	(0.07)
Weight, kg (mean, SD)	70.1	(13.4)
Waist:Hip ratio (mean, SD)	0.79	(0.06)
Parity (n, %)		
Nulliparous	55	(28.6)
Had 1 or 2 children	69	(35.9)
Had 3+ children	65	(33.8)
Had a history of breast-feeding§ (n, %)	111	(82.2)
Had a history of hormone use^T (n, %)	137	(71.4)
First degree relative with breast and/or ovarian cancer (n, %)	24	(12.5)
Smoking status (n, %)		
Current	8	(4.2)
Former	54	(28.1)
Never	128	(66.7)
Race / ethnicity (n, %)		
Asian	14	(7.3)
White	166	(86.5)
Other	10	(5.2)
Years of school completed (n, %)		
≤ 12	12	(6.2)
13 – 15	49	(25.5)
16	57	(29.7)
≥ 17 years	72	(37.5)
Income (n, %)		
≤ $49,999	29	(15.1)
$50,000 - $75,000	44	(22.9)
> $75,000	90	(46.9)
Prefer not to answer	26	(13.5)

§Among parous women only (n = 135)

*Numbers may not add up to 192 for some characteristics due to missing values

^{T}Use of oral contraceptives, hormone patches, hormone injections, hormone implants, or intrauterine devices containing progesterone at any time prior to the 6 month-period before the screening mammogram

Table 2

Mean (SD) and median (range) values and correlation matrices of hormone, binding protein, and urinary estrogen metabolite measures of study population

Hormone[#]	n	Mean	(SD)	Median	(Range)	Correlation matrix											
						E_1	E_1S	E_2	FE_2	A	T	DHEA	DS	FT	SHBG	2-OH E_1	16α-OH E1	2:16α-OH E1
Estrogens																		
E_1 (pg/ml)	189	74.0	(25.6)	71.0	(26 - 205)	1.00												
E_1S (ng/ml)	189	1.6	(0.6)	1.5	(0.5 – 5.1)	0.64	1.00											
E_2 (pg/ml)	189	115	(61.3)	97.0	(15 - 361)	0.69	0.45	1.00										
FE_2 (pg/ml)	189	2.7	(1.5)	2.4	(0.3 – 9.2)	0.55	0.36	0.89	1.00									
Androgens																		
A (ng/ml)	189	0.98	(0.29)	0.95	(0.4 – 2.1)	0.17	0.24	0	0.01	1.00								
T (ng/dl)	189	26.6	(9.2)	25.4	(10 – 60)	0.23	0.13	0.05	0.03	0.68	1.00							
DHEA (ng/ml)	188	4.0	(1.8)	3.8	(0.7 – 13)	0.02	0.25	-0.08	-0.08	0.62	0.52	1.00						
DS (μg/dl)	189	89.3	(47.8)	78.3	(17 – 266)	-0.05	0.34	-0.17	-0.17	0.40	0.34	0.68	1.00					
FT (pg/ml)	189	4.6	(1.8)	4.0	(1 – 12)	0.10	0.12	-0.14	-0.13	0.53	0.79	0.50	0.45	1.00				
SHBG (nmol/l)	189	57.7	(24.4)	55.5	(14 – 188)	0.19	0.03	0.27	0.24	0.14	0.17	-0.01	-0.17	-0.40	1.00			
Urinary catechol estrogen metabolites																		
2-OH E_1 (ng/mg cr)	191	18.7	(13.0)	15.0	(2 – 99)	0.24	0.15	0.32	0.32	0.06	0.09	0.01	0	-0.01	0.17	1.00		
16α-OH E1 (ng/mg cr)	191	11.7	(5.2)	10.6	(4 – 39)	0.16	0.17	0.25	0.22	0.13	0.19	0.22	0.24	0.16	0.07	0.55	1.00	
2:16α-OH E1	191	1.6	(0.9)	1.5	(0.3 – 8)	0.13	0.04	0.17	0.18	0	-0.02	-0.15	-0.20	-0.15	0.17	0.69	-0.13	1.00

[#]E_1 – estrone, E_1S – estrone sulfate, E_2 – estradiol, A – androstenedione, T – testosterone, DHEA – dehydroepiandrosterone, DS - DHEA sulfate, SHBG – sex hormone binding globulin; F = free, OH – hydroxyl, cr – creatinine

Table 3

Adjusted[§] logistic regression analysis: Percent mammographic breast density (highest quartile vs lower quartiles) and quartiles of serum hormones, SHBG, and urinary catechol estrogen metabolites

Hormone[#] (Quartile Cut points)	Median percent density	n[p]	Odds Ratio (95% CI) [η]	p trend
Estrogens				
E_1 (pg/ml)				
1 (≤ 57)	29.5	9/39	Ref	
2 (> 57-71)	38.8	13/33	1.60 (0.54, 4.76)	0.15
3 (> 71-87)	30.6	11/39	1.52 (0.47, 4.94)	
4 (> 87)	35.1	14/30	2.58 (0.76, 8.69)	
E_1S (ng/ml)				
1 (≤ 1.14)	35.4	11/36	Ref	
2 (> 1.14-1.46)	29.9	7/39	0.80 (0.25, 2.52)	0.67
3 (> 1.46-1.94)	51.4	18/30	2.01 (0.74, 5.43)	
4 (> 1.94)	34.0	11/36	0.91 (0.29, 2.80)	
E_2 (pg/ml)[e]				
1 (≤ 68)	30.0	7/40	Ref	
2 (> 68-97)	35.5	10/38	1.36 (0.43, 4.28)	0.08
3 (> 97-152)	35.6	14/35	1.67 (0.57, 4.90)	
4 (> 152)	41.8	16/28	2.87 (0.98, 8.42)	
Androgens				
A (ng/ml)				
1 (≤ 0.79)	26.0	12/33	Ref	
2 (> 0.79-0.95)	29.8	10/41	0.37 (0.12, 1.13)	0.43
3 (> 0.95-1.14)	41.1	10/35	0.50 (0.17, 1.47)	
4 (> 1.14)	38.9	15/32	0.50 (0.17, 1.46)	
T (ng/dl)[e]				
1 (≤ 20.2)	37.0	14/33	Ref	
2 (> 20.2-25.4)	34.6	10/37	0.55 (0.17, 1.78)	0.20
3 (> 25.4-31.4)	35.9	13/34	0.70 (0.24, 2.04)	
4 (> 31.4)	32.8	10/37	0.41 (0.13, 1.30)	
DHEA (ng/ml)				
1 (≤ 2.79)	25.5	8/39	Ref	
2 (> 2.79-3.79)	44.1	14/34	1.60 (0.52, 4.94)	0.68
3 (> 3.79-4.66)	39.2	15/31	1.52 (0.53, 4.35)	
4 (> 4.66)	34.3	10/36	0.84 (0.28, 2.49)	

Hormone[#] (Quartile Cut points)	Median percent density	n[p]	Odds Ratio (95% CI) [η]	p trend
DHEA-S (μg/dl)				
1 (≤ 55.7)	29.8	9/37	Ref	
2 (> 55.7-78.8)	35.9	12/36	1.46 (0.50, 4.28)	0.34
3 (> 78.8-118)	35.6	12/36	1.60 (0.54, 4.74)	
4 (> 118)	46.6	14/32	1.56 (0.54, 4.50)	
Binding protein				
SHBG (nmol/l)[δ]				
1 (≤ 40.8)	20.8	1/46	Ref	
2 (> 40.8-55.4)	30.0	9/38	6.97 (0.98, 49.3)	0.02
3 (> 55.4-71.3)	40.9	16/31	9.84 (1.39, 69.8)*	
4 (> 71.3)	53.7	21/26	11.9 (1.66, 85.0)*	
Urinary catechol estrogen metabolites				
2-OH E_1 (ng/mg creatinine)				
1 (≤ 11.0)	25.1	4/43	Ref	
2 (> 11.0-14.9)	37.0	14/34	3.31 (0.91, 12.1)	0.47
3 (> 14.9-22.3)	48.8	17/30	4.78 (1.36, 16.8)*	
4 (> 22.3)	40.7	12/36	1.96 (0.54, 7.11)	
16α-OH E_1 (ng/mg creatinine)				
1 (≤ 8.13)	26.8	7/41	Ref	
2 (> 8.13-10.6)	39.6	12/35	2.14 (0.63, 7.29)	0.08
3 (> 10.6-14.5)	43.4	14/33	2.15 (0.69, 6.73)	
4 (> 14.5)	39.3	14/34	2.89 (0.94, 8.82)	
2:16α-OH E_1				
1 (≤ 1.14)	23.5	7/40	Ref	
2 (> 1.14-1.49)	39.7	13/35	1.43 (0.44, 4.73)	0.30
3 (> 1.49-2.07)	32.2	9/38	1.01 (0.27, 3.74)	
4 (> 2.07)	45.4	18/30	2.09 (0.63, 6.87)	

[#] E_1 – estrone, E_1S – estrone sulfate, E_2 – estradiol, A – androstenedione, T – testosterone, DHEA – dehydroepiandrosterone, DHEAS – DHEA sulfate, SHBG – sex hormone binding globulin, OH – hydroxy

[p] No. of women in the highest quartile of percent density / No. of women in the lower quartiles of percent density

* $p < 0.05$

[§] adjusted for BMI, number of pregnancies, and history of breastfeeding

additionally adjusted for SHBG;

δ additionally adjusted for E_2 and T

η highest quartile of percent density compared to lower three quartiles of percent density

Table 4

Adjusted[§] logistic regression analysis: Lumbar spine BMD (highest quartile vs lower quartiles) and quartiles of serum hormones and urinary estrogen metabolites

Hormone[#] (Quartile Cut points)	Median lumbar spine (g/cm^2)	n[p]	Odds Ratio (95% CI)[η]	p trend
Estrogens				
E_1 (pg/ml)				
1 (≤ 57)	1.040	12/36	Ref	
2 (> 57-71)	1.058	11/37	1.02 (0.38, 2.73)	0.64
3 (> 71-87)	1.042	12/37	1.00 (0.39, 2.54)	
4 (> 87)	1.045	13/31	1.30 (0.50, 3.37)	
E_1S (ng/ml)				
1 (≤ 1.15)	1.058	15/34	Ref	
2 (> 1.15-1.46)	1.041	9/35	0.51 (0.19, 1.39)	0.95
3 (> 1.46-1.94)	1.038	10/38	0.66 (0.25, 1.77)	
4 (> 1.94)	1.062	14/34	0.98 (0.39, 2.47)	
E_2 (pg/ml)[ef]				
1 (≤ 68)	1.086	14/36	Ref	
2 (> 68-97)	1.043	13/32	1.25 (0.50, 3.12)	0.77
3 (> 97-152)	1.039	7/42	0.54 (0.20, 1.49)	
4 (> 152)	1.014	14/31	1.47 (0.57, 3.78)	
Androgens				
A (ng/ml)				
1 (≤ 0.79)	1.089	17/28	Ref	
2 (> 0.79-0.95)	1.036	7/44	0.32 (0.11, 0.95)*	0.69
3 (> 0.95-1.15)	1.023	10/36	0.60 (0.22, 1.64)	
4 (> 1.15)	1.055	14/33	1.06 (0.40, 2.78)	
T (ng/dl)[ef]				
1 (≤ 20.2)	1.089	18/29	Ref	
2 (> 20.2-25.4)	1.026	9/39	0.40 (0.15, 1.05)	0.32
3 (> 25.4-31.5)	1.038	9/38	0.44 (0.17, 1.19)	
4 (> 31.5)	1.014	12/35	0.61 (0.25, 1.48)	
DHEA (ng/ml)				
1 (≤ 2.82)	1.085	15/32	Reference	
2 (> 2.82-3.79)	1.045	12/36	0.79 (0.30, 2.06)	0.98
3 (> 3.79-4.71)	1.009	5/41	0.31 (0.10, 0.99)*	
4 (> 4.71)	1.069	15/32	1.21 (0.48, 3.03)	

Hormone[#] (Quartile Cut points)	Median lumbar spine (g/cm^2)	n[p]	Odds Ratio (95% CI)[η]	p trend
DHEA-S (μg/dL)				
1 (≤ 55.7)	1.062	12/34	Ref	
2 (> 55.7-78.3)	1.033	14/34	1.24 (0.46, 3.30)	0.97
3 (> 78.3-118)	1.039	9/40	0.57 (0.21, 1.58)	
4 (> 118)	1.039	13/33	1.27 (0.48, 3.36)	
Binding protein				
SHBG (nmol/l)[δ]				
1 (≤ 41.1)	1.090	16/32	Ref	
2 (> 41.1-55.5)	1.019	12/35	1.14 (0.44, 3.00)	0.96
3 (> 55.5-71.2)	1.040	12/35	1.33 (0.45, 3.92)	
4 (> 71.2)	1.021	8/39	0.93 (0.28, 3.09)	
Urinary catechol estrogen metabolites				
2-OH E_1 (ng/mg creatinine)				
1 (≤ 11.0)	1.064	15/32	Ref	
2 (> 11.0-15.0)	1.069	14/35	0.97 (0.39, 2.41)	0.36
3 (> 15.0-22.3)	1.018	8/39	0.53 (0.19, 1.48)	
4 (> 22.3)	1.042	10/38	0.74 (0.28, 2.00)	
16α-OH E_1 (ng/mg creatinine)				
1 (≤ 8.13)	1.066	15/33	Ref	
2 (> 8.13-10.6)	1.062	13/34	0.90 (0.36, 2.25)	0.25
3 (> 10.6-14.5)	1.036	9/39	0.55 (0.21, 1.46)	
4 (> 14.5)	1.026	10/38	0.64 (0.24, 1.70)	
2:16α-OH E_1				
1 (≤ 1.14)	1.061	13/34	Ref	
2 (> 1.14-1.49)	1.069	15/34	1.41 (0.56, 3.52)	0.60
3 (> 1.49-2.07)	1.037	9/38	0.70 (0.26, 1.89)	
4 (> 2.07)	1.035	10/38	0.98 (0.37, 2.60)	

[#] E_1 – estrone, E_1S – estrone sulfate, E_2 – estradiol, A – androstenedione, T – testosterone, DHEA – dehydroepiandrosterone, DHEAS – DHEA sulfate, SHBG – sex hormone binding globulin, OH – hydroxy

[p] No. of women in the highest quartile of BMD / No. of women in the lower quartiles of BMD

[*] $p < 0.05$

[§] adjusted for BMI

additionally adjusted for SHBG

[δ] additionally adjusted for E_2 and T

$^{\eta}$highest quartile of BMD compared to lower three quartiles of BMD

Table 5

Adjusted[§] logistic regression analysis: Pelvic BMD (highest quartile vs lower quartiles) and quartiles of serum hormones and urinary estrogen metabolites

Hormone[#] (Quartile Cut points)	Median pelvic BMD (g/cm^2)	n[p]	Odds Ratio (95% CI)[η]	p trend
Estrogens				
E_1 (pg/ml)				
1 (≤ 57)	1.168	16/32	Ref	
2 (> 57-71)	1.138	7/41	0.38 (0.13, 1.12)	0.73
3 (> 71-87)	1.145	13/36	0.71 (0.28, 1.79)	
4 (> 87)	1.141	12/32	0.72 (0.26, 2.03)	
E_1S (ng/ml)				
1 (≤ 1.15)	1.138	12/37	Ref	
2 (> 1.15-1.46)	1.158	14/30	1.31 (0.46, 3.72)	0.81
3 (> 1.46-1.94)	1.147	9/39	0.89 (0.30, 2.63)	
4 (> 1.94)	1.130	13/35	1.29 (0.47, 3.57)	
E_2 (pg/ml)[e]				
1 (≤ 68)	1.189	18/32	Ref	
2 (> 68-97)	1.156	11/34	0.80 (0.30, 2.14)	0.85
3 (> 97-152)	1.136	5/44	0.30 (0.09, 1.00)	
4 (> 152)	1.134	14/31	1.45 (0.54, 3.89)	
Androgens				
A (ng/ml)				
1 (≤ 0.79)	1.198	18/27	Reference	
2 (> 0.79-0.95)	1.141	9/42	0.46 (0.15, 1.40)	0.83
3 (> 0.95-1.15)	1.139	9/37	0.58 (0.20, 1.66)	
4 (> 1.15)	1.130	12/35	1.07 (0.38, 2.98)	
T (ng/dl)[e]				
1 (≤ 20.2)	1.152	14/33	Reference	
2 (> 20.2-25.4)	1.145	11/37	0.84 (0.30, 2.37)	0.46
3 (> 25.4-31.5)	1.138	10/37	0.98 (0.34, 2.86)	
4 (> 31.5)	1.133	13/34	1.42 (0.52, 3.90)	
DHEA (ng/ml)				
1 (≤ 2.82)	1.162	14/33	Reference	
2 (> 2.82-3.79)	1.137	9/39	0.62 (0.21, 1.84)	0.27
3 (> 3.79-4.71)	1.114	9/37	0.84 (0.26, 2.73)	
4 (> 4.71)	1.161	15/32	1.67 (0.61, 4.57)	

Hormone[#] (Quartile Cut points)	Median pelvic BMD (g/cm^2)	n[p]	Odds Ratio (95% CI)[η]	p trend
DHEA-S (μg/dL)				
1 (≤ 55.7)	1.166	11/35	Reference	
2 (> 55.7-78.3)	1.129	14/34	1.53 (0.52, 4.53)	0.58
3 (> 78.3-118)	1.138	9/40	0.55 (0.18, 1.65)	
4 (> 118)	1.144	14/32	1.89 (0.67, 5.35)	
Binding protein				
SHBG (nmol/l)[δ]				
1 (≤ 41.1)	1.217	23/25	Reference	
2 (> 41.1-55.5)	1.162	11/36	0.62 (0.24, 1.61)	0.22
3 (> 55.5-71.2)	1.136	7/40	0.40 (0.12, 1.31)	
4 (> 71.2)	1.119	7/40	0.50 (0.15, 1.71)	
Urinary catechol estrogen metabolites				
2-OH E_1 (ng/mg creatinine)				
1 (≤ 11.0)	1.170	16/31	Ref	
2 (> 11.0-15.0)	1.142	17/32	1.37 (0.53, 3.55)	0.06
3 (> 15.0-22.3)	1.133	8/39	0.55 (0.18, 1.64)	
4 (> 22.3)	1.125	6/42	0.43 (0.14, 1.37)	
16α-OH E_1 (ng/mg creatinine)				
1 (≤ 8.13)	1.144	15/33	Ref	
2 (> 8.13-10.6)	1.163	10/37	0.64 (0.23, 1.77)	0.84
3 (> 10.6-14.5)	1.132	10/38	0.64 (0.23, 1.77)	
4 (> 14.5)	1.137	12/36	0.90 (0.32, 2.53)	
2:16α-OH E_1				
1 (≤ 1.14)	1.179	19/28	Ref	
2 (> 1.14-1.49)	1.152	14/35	0.76 (0.30, 1.95)	0.03
3 (> 1.49-2.07)	1.141	7/40	0.27 (0.09, 0.84)[*]	
4 (> 2.07)	1.116	7/41	0.41 (0.14, 1.16)	

[#] E_1 – estrone, E_1S – estrone sulfate, E_2 – estradiol, A – androstenedione, T – testosterone, DHEA – dehydroepiandrosterone, DHEAS – DHEA sulfate, SHBG – sex hormone binding globulin, OH – hydroxy

[p] No. of women in the highest quartile of BMD / No. of women in the lower quartiles of BMD

[*] $p < 0.05$

[§] adjusted for BMI

additionally adjusted for SHBG

[δ] additionally adjusted for E_2 and T

$^{\eta}$highest quartile of BMD compared to lower three quartiles of BMD

Table 6

Adjusted[§] logistic regression analysis: Head BMD (highest quartile vs lower quartiles) and quartiles of serum hormones and urinary estrogen metabolites

Hormone[#] (Quartile Cut points)	Median head BMD (g/cm^2)	n[p]	Odds Ratio (95% CI)[η]	p trend
Estrogens				
E_1 (pg/ml)				
1 (≤ 57)	2.299	14/34	Ref	
2 (> 57-71)	2.305	11/37	0.75 (0.30, 1.87)	0.81
3 (> 71-87)	2.332	11/38	0.71 (0.28, 1.77)	
4 (> 87)	2.409	12/32	0.92 (0.37, 2.27)	
E_1S (ng/ml)				
1 (≤ 1.15)	2.320	12/37	Ref	
2 (> 1.15-1.46)	2.348	13/31	1.26 (0.50, 3.17)	0.69
3 (> 1.46-1.94)	2.239	13/35	1.18 (0.47, 2.96)	
4 (> 1.94)	2.390	10/38	0.82 (0.32, 2.15)	
E_2 (pg/ml)[e]				
1 (≤ 68)	2.350	12/38	Ref	
2 (> 68-97)	2.282	12/33	1.27 (0.50, 3.23)	0.50
3 (> 97-152)	2.386	12/37	1.26 (0.50, 3.18)	
4 (> 152)	2.308	12/33	1.42 (0.54, 3.72)	
Androgens				
A (ng/ml)				
1 (≤ 0.79)	2.403	16/29	Reference	
2 (> 0.79-0.95)	2.269	12/39	0.58 (0.23, 1.44)	0.19
3 (> 0.95-1.15)	2.263	10/36	0.52 (0.20, 1.37)	
4 (> 1.15)	2.346	10/37	0.52 (0.20, 1.35)	
T (ng/dl)[e]				
1 (≤ 20.2)	2.450	18/29	Reference	
2 (> 20.2-25.4)	2.305	15/33	0.72 (0.31, 1.70)	0.01
3 (> 25.4-31.5)	2.237	7/40	0.29 (0.10, 0.80)*	
4 (> 31.5)	2.295	8/39	0.36 (0.14, 0.94)*	
DHEA (ng/ml)				
1 (≤ 2.82)	2.355	13/34	Reference	
2 (> 2.82-3.79)	2.284	15/33	1.24 (0.51, 3.02)	0.19
3 (> 3.79-4.71)	2.286	11/35	0.88 (0.34, 2.27)	
4 (> 4.71)	2.320	8/39	0.56 (0.21, 1.53)	

Hormone[#] (Quartile Cut points)	Median head BMD (g/cm^2)	n[p]	Odds Ratio (95% CI)[η]	p trend
DHEA-S (μg/dL)				
1 (≤ 55.7)	2.343	13/33	Reference	
2 (> 55.7-78.3)	2.385	13/35	0.95 (0.38, 2.37)	0.29
3 (> 78.3-118)	2.356	14/35	1.00 (0.41, 2.44)	
4 (> 118)	2.226	8/38	0.55 (0.20, 1.49)	
Binding protein				
SHBG (nmol/l)[δ]				
1 (≤ 41.1)	2.394	14/34	Reference	
2 (> 41.1-55.5)	2.356	14/33	1.22 (0.47, 3.15)	0.22
3 (> 55.5-71.2)	2.302	14/33	1.23 (0.44, 3.47)	
4 (> 71.2)	2.282	6/41	0.46 (0.13, 1.63)	
Urinary catechol estrogen metabolites				
2-OH E_1 (ng/mg creatinine)				
1 (≤ 11.0)	2.403	12/35	Ref	
2 (> 11.0-15.0)	2.282	12/37	1.00 (0.39, 2.54)	0.94
3 (> 15.0-22.3)	2.302	13/34	1.21 (0.48, 3.08)	
4 (> 22.3)	2.272	11/37	0.97 (0.36, 2.57)	
16α-OH E_1 (ng/mg creatinine)				
1 (≤ 8.13)	2.365	12/36	Ref	
2 (> 8.13-10.6)	2.295	13/34	1.18 (0.47, 2.93)	0.94
3 (> 10.6-14.5)	2.320	11/37	0.93 (0.37, 2.35)	
4 (> 14.5)	2.265	12/36	1.04 (0.42, 2.60)	
2:16α-OH E_1				
1 (≤ 1.14)	2.364	12/35	Ref	
2 (> 1.14-1.49)	2.386	12/37	1.01 (0.39, 2.62)	0.83
3 (> 1.49-2.07)	2.276	12/35	1.05 (0.41, 2.70)	
4 (> 2.07)	2.281	12/36	1.10 (0.41, 2.93)	

[#] E_1 – estrone, E_1S – estrone sulfate, E_2 – estradiol, A – androstenedione, T – testosterone, DHEA – dehydroepiandrosterone, DHEAS – DHEA sulfate, SHBG – sex hormone binding globulin, OH – hydroxy

[p] No. of women in the highest quartile of BMD / No. of women in the lower quartiles of BMD

[*] $p < 0.05$

[§] adjusted for BMI

additionally adjusted for SHBG

[δ] additionally adjusted for E_2 and T

$^{\eta}$highest quartile of BMD compared to lower three quartiles of BMD