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Serum factors and clinical characteristics associated with serum E-Screen activity

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Abstract

Background—The E-Screen bioassay can measure the mitogenicity of human serum and thus may be useful as a biomarker in epidemiologic studies of breast cancer. While the assay's MCF-7 cells are known to proliferate in response to estrogen, the specific determinants of variation in E-Screen activity in human serum samples are poorly understood. We sought to identify serum molecules and patient characteristics associated with serum E-Screen activity among postmenopausal women.

Methods—Postmenopausal women (N=219) aged 55–70 with no history of postmenopausal hormone use or breast cancer completed a questionnaire and provided a blood sample. Serum was analyzed for E-Screen activity and a variety of molecules including sex hormones, growth factors, and environmental chemicals. Stepwise selection procedures were used to identify correlates of E-Screen activity.

Results—Serum samples from all women had detectable E-Screen activity, with a median estradiol equivalents value of 0.027 ng/mL and interquartile range of 0.018–0.036 ng/mL. In the final multivariable-adjusted model, serum E-Screen activity was positively associated with serum estradiol, estrone, IGFBP-3, and testosterone levels ($p < 0.05$), as well as body mass index ($p = 0.03$). Serum E-Screen activity was lower among women with higher SHBG ($p < 0.0001$) and progesterone levels ($p = 0.03$).

Conclusion—Serum E-Screen activity varies according to levels of endogenous estrogens and other serum molecules. Obesity appears to confer additional serum mitogenicity beyond its impact on the measured hormones and growth factors.

Impact—By capturing mitogenicity due to a variety of patient and serum factors, the E-Screen may provide advantages for use as a biomarker in breast cancer studies.

Keywords

E-Screen activity; estradiol equivalents; breast cancer; MCF-7; epidemiology

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

Introduction

Epidemiological research on breast cancer risk often requires long term follow-up of patients. The use of an intermediate marker of breast cancer risk could allow smaller, less expensive, and quicker studies. The development of novel biomarkers is clearly needed to improve the study of breast cancer risk (1).

One potential biomarker for breast cancer risk is serum E-Screen activity. The E-Screen bioassay measures the proliferation of MCF-7 breast cancer cells in response to test samples that are added to the cell culture media. By measuring the mitogenic capacity of human serum samples, the E-Screen bioassay could potentially be used to predict breast cancer risk or to evaluate the impact of an intervention designed to reduce breast cancer risk.

MCF-7 cells are known to proliferate in response to estrogen (2) and estrogen levels have been shown to be predictive of breast cancer risk (3–5). However, a large number of other endogenous and exogenous molecules influence MCF-7 cell proliferation. The E-Screen bioassay has been used extensively to measure the estrogenic activity of environmental chemicals (6, 7), with a substantial literature documenting a large number of identified estrogen-mimicking “xenoestrogens” (8, 9). In addition to estradiol, a number of endogenous sex hormones (including estrone and testosterone) influence MCF-7 cell proliferation in the E-Screen bioassay (10). Thus, the mitogenic activity of human serum as measured in the E-Screen bioassay likely reflects the combined effect of numerous endogenous and exogenous mitogens. By providing a comprehensive measure of serum mitogenicity, the E-Screen bioassay may offer advantages as a biomarker compared to the measurement of estradiol or other specific serum molecules.

To evaluate the potential for using the serum E-Screen activity as a breast cancer biomarker, a better understanding of the determinants of variation in E-Screen activity in human serum is needed. The purpose of this study was to identify serum molecules and patient characteristics associated with serum E-Screen bioassay activity. We evaluated a number of circulating endogenous and exogenous serum molecules as well as patient factors known to be associated with breast cancer risk, including body mass index, alcohol consumption, and mammographic breast density.

METHODS

Study population

The study population consisted of women enrolled in the Wisconsin Breast Density Study (11, 12), which was approved by the University of Wisconsin Health Sciences Institutional Review Board. Details regarding subject recruitment have been previously described (11). Briefly, eligibility was limited to postmenopausal women (defined as no menstrual cycles within the past 12 months) aged 55–70 years receiving a screening mammogram at two clinics in Madison, Wisconsin. Women were excluded if they had ever used postmenopausal hormones or tamoxifen, had breast implants, or had a personal history of breast cancer. A total of 268 women were enrolled between June 2008 and July 2009.

Questionnaire

All subjects completed a questionnaire which included factors potentially related to breast cancer risk, including age, age at menopause, weight, height, first degree family history of breast cancer, parity, alcohol consumption, smoking, physical activity, lactation history, and education level. Alcohol consumption was assessed as the typical number of drinks of beer, wine, or hard liquor consumed per day, week, or month. For women reporting that they had smoked more than 100 cigarettes in their lifetime we obtained the age at smoking initiation,

age at smoking cessation (if no longer smoking), and the average number of cigarettes smoked per day over the duration of her smoking history. Physical activity was assessed as the average number of hours per week engaged in physically vigorous activities that cause large increases in heart rate or breathing (e.g., jogging, swimming laps, etc.). Lactation history was measured as the total number of months for which a woman breastfed her children.

Mammographic breast density

All subjects underwent their scheduled mammogram and mammographic breast density was quantitatively assessed as previously described (11, 12). Briefly, digital images of the craniocaudal view of the left breast were obtained and percent breast density was measured by a computer-aided thresholding method using Cumulus software (13).

Quantification of circulating serum molecules

A whole blood sample of up to 30 mL was collected via venipuncture in uncoated glass red top vacutainer tubes (Fisher Scientific, Pittsburgh, PA) and was allowed to clot for 30 minutes, then spun down for 20 minutes in a centrifuge at 2500 rpm. Single aliquots of 4.5 mL were transferred into borosilicate glass vials for the analyses of E-Screen activity and exogenous chemical levels (phytoestrogens, phthalates, parabens, and phenols). The glass vials were prepared by baking at 450 degree Celsius to burn off all organic carbon and the Teflon-coated caps were sonicated in methanol to remove any contaminants. Additional serum was distributed in 2 mL aliquots into plastic cryovials for the analyses of sex hormones and other endogenous molecules. All samples were immediately frozen at -70°C and thawed at the laboratories for analysis.

Details on the methods used for the quantification of sex hormones and other circulating endogenous molecules in this study, as well as assay reliability and quality control, have been previously described (11, 12). Briefly, sex hormone analyses and quantification of 25-hydroxyvitamin D (25(OH)D), IGF-1, and IGFBP-3 were conducted at the Reproductive Endocrine Research Laboratory at the University of Southern California. Progesterone, testosterone, estrone, and estradiol were quantified by validated, previously described radioimmunoassays (RIAs) which included purification steps to improve specificity (14–16). Prior to the RIA, the steroids were extracted from serum with hexane:ethyl acetate. The steroids were then separated by Celite column partition chromatography using trimethylpentane, toluene in trimethylpentane, and ethyl acetate in trimethylpentane. Sex hormone binding globulin (SHBG) was quantified by direct chemiluminescent immunoassay using the Immulite analyzer (Siemens Medical Solutions Diagnostics, Malvern, Pennsylvania) and the inter-assay coefficients of variation (CV) ranged between 7% and 12%. 25(OH)D was measured using a commercial ^{125}I -based RIA kit (DiaSorin, Stillwater, MN), with a preliminary organic solvent extraction step. IGF-1 and IGFBP-3 were quantified by direct chemiluminescent immunoassays using the Immulite analyzer (Siemens Medical Solutions Diagnostics, Malvern, PA). Previous studies using the DiaSorin kit have reported excellent intra-assay and inter-assay CVs of $<10\%$ (17, 18).

Parathyroid hormone (PTH), calcium, and retinol were analyzed at the University of Wisconsin Carbone Cancer Center's Analytical Instrumentation Laboratory for Pharmacokinetics, Pharmacodynamics, and Pharmacogenetics. Intact PTH was measured by a two-site Enzyme-Linked Immunosorbent Assay (ELISA; MD Biosciences, St. Paul, MN), which has previously been shown to have intra-assay and inter-assay CVs of less than 4% (19, 20). Calcium was measured by quantitative colorimetric determination using the Quanti-Chrom calcium assay kit (Bioassay Systems, Hayward, CA). Retinol was quantified

by HPLC, as described by Taibi and Nicotra (21) who reported intra-assay and inter-assay CVs of less than 3.5%.

A panel of estrogenically active phytoestrogens, phthalates, parabens, and phenols to which humans are routinely exposed were selected for serum analyses. Genistein, daidzein, coumestrol, mono-ethyl phthalate, propyl paraben, butyl paraben, bisphenol A, nonylphenol, and octylphenol were evaluated at the Wisconsin State Laboratory of Hygiene using solid phase extraction (Strata-X, Phenomenex, Torrance, CA) and isotope dilution high-performance liquid chromatography (Agilent 1100, Waldbronn, Germany) with tandem mass spectrometry (API4000, AB/SCIEX, Framingham, MA) with APCI negative ionization (22–25). Analytical quality assurance (QA) parameters included reagent (all <LOD) and method blanks (all <LOD with exception of nonylphenol, for which 5 of 9 were >LOD), calibration check standards (recovery = 98.7% to 114.1%; n=31 for phthalates, parabens, and phytoestrogens and n=20 for phenols), and double charcoal treated human serum matrix control spikes at low (1 ng/mL, recovery = 82.9% to 114%; n=12 for phthalates, parabens, and phytoestrogens and n=14 for phenols) and mid (5 and 10 ng/mL, recovery = 87.4% to 112.9%; n=12 for phthalates and parabens, n=31 for phytoestrogens, and n=19 for phenols) calibration curve levels.

E-Screen bioassay

The serum samples were evaluated for mitogenic activity by the E-Screen, a bioassay that compares the proliferation of MCF-7 cells in the presence of test substances to the proliferation achieved in response to a standard curve of estradiol (6, 26). The cell line was obtained from Drs. Ana Soto and Carlos Sonnenschein at Tufts University School of Medicine in March 2000. Tests for cell line authentication include thrice-weekly morphology check by microscope, growth curve analysis in relation to standard estradiol concentrations with every E-Screen run, and periodic assays to detect mycoplasma. To measure E-Screen activity, MCF-7 cells were plated at 25,000 cells per well in 24-well plates and incubated for 24 hours before changing to fresh medium containing 5% human serum samples (study samples) or commercially available charcoal-dextran (CD) stripped human serum (negative control). Samples were run concurrently with a standard curve of 15 concentrations of 17 β -estradiol in 5% CD human serum. After a five-day incubation, cells were fixed, stained and re-suspended in buffer and absorbance was read on a plate-reader. Evidence for the lack of estrogenic activity of the negative control included the low cellular proliferation in response to CD-stripped human serum alone, which was not higher than the cellular proliferation observed using CD-stripped fetal bovine serum alone. The single batch of CD-stripped human serum used in these assays resulted in equivalent cellular proliferation to that observed with prior batches of CD-stripped fetal bovine serum (historical controls). Furthermore, the addition of CD-stripped human serum did not shift the estradiol standard curve. The values for the study samples were compared to the standard curve to determine the proliferation relative to the 17 β -estradiol standards, and were reported as 17 β -estradiol equivalents (EEq) in ng/mL serum. Each serum sample from an individual study subject was tested in triplicate and the mean of these measurements was used for data analysis. To assess the assay reliability, a duplicate serum sample was provided for 10% of study subjects (N=23). The intra-class correlation coefficient between values for duplicate samples was 0.94, indicating high reliability.

Statistical analyses

The analyses were restricted to the 219 women who had sufficient serum available for the E-Screen bioassay. Spearman correlation coefficients were calculated to assess the association between each circulating serum molecule and serum estradiol, since estradiol is known to be the most potent endogenous hormone in the E-Screen bioassay. Quantification of PTH,

retinol, calcium and IGF-1 were missing for 15 women and certain covariate data were missing for a small fraction (<5%) of subjects (see Table 1). To enable the most statistically efficient use of all the data, multiple imputation was used to impute missing covariate data, with 100 imputations conducted using the Markov Chain Monte Carlo method (27). Stepwise model selection was applied separately for a multivariable linear regression model in each of the 100 imputed datasets to identify serum molecules and patient characteristics associated with serum E-Screen activity. To improve normality, E-Screen values were transformed using the logarithm function. For the stepwise model selection process, we used Akaike information criterion (AIC) (28, 29) as the criterion for selecting and eliminating a variable. Variables that were evaluated for inclusion in the stepwise model included all available patient clinical factors and serum measurements known or suspected to be related to breast cancer risk, breast cancer cell proliferation, estrogen metabolism, or endocrine disruption. Table 1–Table 3 list all evaluated variables. Family history of breast cancer, parity, lactation history, weight gain, alcohol consumption, smoking, vigorous physical activity and education level were included as categorical variables in the model. All other variables were evaluated as continuous terms in statistical models.

Results from the 100 imputed datasets were summarized by the frequency with which each variable was included in the model produced by the stepwise process (30). A final multivariable linear regression model was established that contained variables that were included in the stepwise process in at least 70 of the 100 imputed datasets (31). This final model was fit separately to the 100 imputed datasets and the results combined for statistical inferences using the methods of Rubin (32). In order to illustrate the difference in serum E-Screen activity according to predictor levels, adjusted least-squares mean levels of the logarithm of serum E-Screen activity were calculated according to quartiles or categories of circulating molecule levels and patient characteristics. These mean values were then reverse transformed for display purposes. Tests of trends across hormone quartile groups or characteristic categories were conducted by inclusion of hormone quartile or characteristic category as an ordinal term in the regression models. All statistical analyses were performed using SAS Statistical Software (Version 9.2; SAS Institute, Inc., Cary, North Carolina).

RESULTS

The average age of participants was 60.7 years of age. Approximately 24% of participants had a first degree family history of breast cancer (Table 1). More than 30% were overweight (BMI 25–29.9 kg/m²) and 34% were obese (BMI ≥ 30 kg/m²). About one-third abstained from alcohol consumption and about 60% had never smoked.

Figure 1 shows the distribution of E-Screen activity, which had a mean value of 0.036 ng/mL and standard deviation of 0.039 ng/mL, but was skewed in a positive direction. The median EEq value was 0.027 ng/mL, with an interquartile range of 0.018–0.036 ng/mL. After logarithm transformation, E-Screen activity more closely resembled a normal distribution.

The distributions of measured endogenous circulating serum molecules are displayed in Table 2. Since estradiol is considered to be the most potent mitogen in the E-Screen bioassay, we examined the association between estradiol and the other serum measurements. Serum estradiol values had a strong positive correlation with serum estrone and testosterone. There were moderate positive correlations between estradiol and progesterone and PTH, and moderate negative correlations between estradiol and SHBG, IGF-1, and retinol. No statistically significant correlations were detected between estradiol and IGFBP-3, calcium and 25(OH)D. Table 3 shows the distribution of serum xenoestrogens and phytoestrogens. There was a moderate positive correlation between estradiol and nonylphenol. No other

statistically significant correlations were detected between estradiol and the other xenoestrogens and phytoestrogens of interest.

Variables which were selected for inclusion in the stepwise models of E-screen activity in more than 70 of the 100 imputed datasets were included in the final multivariable model of E-Screen activity. Estradiol, estrone, SHBG, and IGFBP-3 were selected for model inclusion in all 100 imputed datasets. BPA, progesterone, testosterone, Retinol, BMI, alcohol consumption and IGF-1 were included in at least 71 of the 100 models. Seven additional variables (coumestrol, breast density, octylphenol, education, 25(OH)D, weight gain within the past year, and weight gain since age 18) were included in fewer than 61 of the stepwise models and were not incorporated into the final multivariable model of E-Screen activity. The remaining variables in Table 1–Table 3 were not selected for inclusion in any of the 100 models.

Table 4 and Figure 2 display the multivariable-adjusted association between each variable and E-Screen activity. E-Screen activity was higher among serum samples with higher estradiol, estrone, IGFBP-3, and testosterone levels (Table 4; $p < 0.05$). E-Screen activity was lower among women with higher SHBG ($p < 0.0001$) and progesterone levels ($p = 0.03$). The associations between E-Screen activity and IGF-1 ($p = 0.08$), retinol ($p = 0.10$), alcohol consumption ($P\text{-trend} = 0.11$), and BPA ($P\text{-trend} = 0.17$) were not statistically significant in the final model. E-Screen activity varied most widely according to quartiles of estrone, with mean EEq values of 0.040 ng/mL (95% CI: 0.036, 0.044 ng/mL) for women in the highest quartile of estrone, compared to 0.021 ng/mL (95% CI: 0.019, 0.022 ng/mL) among women in the lowest quartile.

There was a positive association between BMI and E-Screen activity (beta coefficient = 0.008; $p = 0.03$). The mean EEq value ranged from 0.026 ng/mL (95% CI: 0.024, 0.028 ng/mL) for women with a BMI less than 25 kg/m² to 0.030 ng/mL (95% CI: 0.028, 0.032 ng/mL) for obese women (Figure 2; $P_{\text{trend}} = 0.01$). There was no statistically significant association between E-Screen activity and alcohol consumption or serum BPA in the final multivariable-adjusted model.

DISCUSSION

We sought to determine which components of the complex mixture of molecules in serum are associated with variation in serum E-Screen activity among healthy postmenopausal women. Our results indicate that serum E-Screen activity is strongly associated with endogenous estrogen levels. However, other circulating molecules and BMI are also predictors of serum E-Screen activity, independent of endogenous estrogens. These results indicate that a number of factors in human serum influence the proliferation of human breast cancer cells as measured by the E-Screen bioassay. Thus, compared to measuring endogenous estrogens alone, the E-Screen bioassay may potentially provide additional information related to breast cancer risk.

We hypothesized that estradiol would be most strongly associated with E-Screen activity since it is the most potent endogenous molecule when tested singly in the E-Screen bioassay (10). However, the range of EEq values appeared to vary more greatly according to quartiles of estrone than for quartiles of estradiol. There may be a number of reasons for these results. Compared to estradiol, serum estrone levels are higher and more widely distributed. The interquartile range for estrone was about 20 pg/mL, compared to approximately 7 pg/mL for estradiol. While estradiol is able to promote E-Screen activity at lower concentrations, the relatively small levels and narrow distribution in postmenopausal women may somewhat limit its influence on inter-person variation in E-Screen activity.

Serum SHBG was inversely associated with E-Screen activity, which is consistent with its known role of inhibiting endogenous estrogen activity (10). Testosterone levels were positively associated with E-screen activity, in agreement with previous studies demonstrating that testosterone alone promotes MCF-7 cell proliferation, likely via the intracellular conversion of testosterone to estradiol by aromatase enzymes (33). Progesterone is inactive when tested individually in the E-Screen assay (34), and there is little evidence available to date to support an association between circulating progesterone levels and breast cancer risk (35). Our finding of an inverse association between circulating progesterone levels and E-Screen activity suggests a need for further investigation of potential biological mechanisms.

A previous study investigating individual endogenous and exogenous molecules found that human recombinant IGF-1 did not affect the proliferation of MCF-7 cells (6). However, we observed a strong positive association between IGFBP-3 and E-Screen activity ($\beta = 0.15$, $P < .0001$), and a negative association between IGF-1 and E-Screen activity that was of borderline statistical significance ($\beta = -0.001$, $P = 0.08$). This result is somewhat surprising given that greater IGF-1 levels are suspected to increase breast cancer risk (36, 37). However, the mechanisms relating the IGF-1 pathway to breast cancer risk are complex, and the influence of IGF-1 on MCF-7 cell proliferation appears to be modified by other endogenous molecules, including estradiol (38).

We hypothesized that BMI may be positively related to E-Screen activity because of its association with elevated endogenous hormone levels (39), but we expected that this association would be eliminated after adjusting for serum sex hormones. However, BMI remained associated with E-Screen activity after controlling for estradiol and other circulating molecules. This finding suggests that other unmeasured serum factors (e.g., other hormones or growth factors) associated with BMI may influence breast cancer cell proliferation.

Alcohol consumption is a risk factor for breast cancer and thus we hypothesized that it would be positively associated with E-Screen activity (40). In contrast we found that E-Screen activity was lower among women with the highest alcohol consumption levels, although the relation was modest and confidence intervals overlapped ($P\text{-trend}=0.11$). Alcohol consumption is believed to increase breast cancer risk via its effects on endogenous hormone metabolism, though the impact of alcohol consumption on serum estradiol and estrone levels is not entirely clear (41). We adjusted for a variety of hormones in our analyses and would have expected any residual confounding by alcohol's effects on hormone metabolism to result in an apparent positive association between alcohol consumption and E-Screen activity. It is possible that the modest observed inverse association may be due to chance, or it may suggest mechanisms of action independent of the measured hormone pathways.

Though BPA was chosen by the stepwise model selection process in 97 imputed datasets, the association between BPA and E-Screen activity was not statistically significant in the final multi-variable adjusted model ($P\text{-trend}=0.17$). Additionally we found no evidence that serum levels of any other xenoestrogens or phytoestrogens were associated with E-Screen activity after adjusting for the endogenous estrogens. While it remains possible that the cumulative effect of serum xenoestrogens may influence the mitogenicity of human serum, the contributions of the individual chemicals we measured appeared to be small.

A handful of previous studies have used HPLC fractionation to separate the endogenous hormone components of human samples from the exogenous chemicals and have examined the estrogenic activity of these fractions separately (42–45). These studies have mainly

characterized the distribution of E-Screen activity of each fraction and identified environmental chemicals present in the exogenous fraction. To our knowledge, this is the first study to examine the E-Screen activity of unfractionated human serum in relation to circulating molecules and patient characteristics. One recent study evaluated the E-Screen activity of unfractionated adipose tissue in women aged 46–60 years old (46). Adipose tissue E-Screen activity was not associated with BMI, but there was a moderate positive association with use of postmenopausal hormones and a weak negative association with age. Our study was restricted to women who had never used postmenopausal hormones so we were unable to evaluate this factor. There was no association between age and serum E-Screen activity in our study. The differences in our results likely reflect the specialized role of adipose tissue in estrogen synthesis among postmenopausal women.

Certain limitations should be considered in the interpretation of this study. Since all women were recruited at screening mammography clinics in Madison, Wisconsin, the results of this study may not be generalizable to other more diverse populations (97% of study subjects reported white race). Additionally, a substantial percentage of women had non-detectable phytoestrogen and xenoestrogen levels, which resulted in limited power to detect differences in serum E-Screen activity according to these molecules. Notably, measurement of estrogen levels in postmenopausal women is difficult due to low levels and cross-reactivity of estrogen metabolites with antibodies used in the RIA assays (47). We used well validated RIAs with preceding purification steps that have been shown to be sensitive, precise, specific, and accurate (47).

Multiple imputation was used to impute the small numbers of missing covariate data. Sensitivity analyses in which subjects with missing values were excluded revealed a negligible impact upon the results. Stepwise model selection was applied to each imputed dataset ($N = 100$), and the inclusion frequency was used as a criteria to determine the final model. We used 70% as the threshold for the inclusion frequency; previous authors have recommended threshold values ranging between 60–90% (31).

The E-screen may represent an attractive candidate for use as a biomarker in studies of breast cancer prevention. Recent studies have demonstrated that combining information regarding circulating levels of a number of hormones can lead to better breast cancer risk stratification (48, 49). In effect, the E-Screen integrates information from all molecules in the blood as it measures mitogenic activity, and thus has the potential to further improve breast cancer risk prediction. An assay similar to E-Screen has been used in clinical trials of prostate cancer, where serum promotion of LNCaP prostate cancer cell proliferation has been used as an outcome marker in trials of dietary interventions among men with rising prostate-specific antigen values (50) and men with prostate cancer (51). Studies of breast cancer risk in relation to serum E-Screen activity are needed to further develop the E-Screen as an intermediate marker. One provocative study reported that the E-Screen activity of the exogenous xenoestrogen fraction of human adipose tissue is associated with an increased risk for breast cancer (52). However, it is also important to recognize that the E-Screen is a single breast cancer cell line and that different breast cancer cell lines are likely to vary in response to the same serum samples. The heterogeneity of breast cancer should be considered in any future efforts to use cell-based assays as biomarkers in cancer prevention and risk prediction.

The results of our study indicate that a number of circulating molecules in human serum are associated with E-Screen activity. Additionally, serum from patients with higher body mass index tended to have higher E-Screen activity, independent of sex hormone levels. These results suggest that E-Screen activity is more than a surrogate for endogenous estrogen

levels, and may potentially be useful as a marker of serum mitogenicity in studies of breast cancer prevention.

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ABBREVIATIONS

EEq	estradiol equivalents
MCF-7	Michigan Cancer Foundation-7
SHBP	sex hormone binding globulin
BMI	body mass index
IGFBP-3	insulin-like growth factor binding protein-3
IGF-1	insulin-like growth factor 1
BPA	bisphenol A
PTH	Parathyroid hormone
25(OH)D	25-hydroxyvitamin D

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Figure 1. Distribution of serum E-Screen activity (panel A) and the log transform of serum E-Screen activity (panel B) among postmenopausal women, Wisconsin Breast Density Study, 2008–2009.

Figure 2.

Multivariable-adjusted mean serum E-Screen activity according to body mass index (BMI; panel A), alcohol consumption (panel B), and bisphenol A (BPA; panel C) levels among postmenopausal women, Wisconsin Breast Density Study, 2008–2009. The mean values are adjusted for estradiol (continuous), estrone (continuous), SHBG (continuous), IGFBP-3 (continuous), testosterone (continuous), progesterone (continuous), IGF-1 (continuous), retinol (ng/mL), BMI (<25, 25–29, ≥ 30 kg/m²), alcohol consumption (0, <5, ≥ 5 drinks per week), and BPA (non-detectable, less than median detected value, ≥ median detected value).

Table 1

Characteristics of study participants (N=219), Wisconsin Breast Density Study, 2008–2009.

	N (%)
Age (years)	
55–59	114 (52.1)
60–64	60 (27.4)
65–70	45 (20.6)
Family history of breast cancer	
No	167 (76.3)
Yes	52 (23.7)
Breast density	
0–6.04%	55 (25.1)
6.04–11.80%	54 (24.7)
11.80–22.30%	55 (25.1)
22.30–71.25%	55 (25.1)
Parity	
0	55 (25.1)
1	29 (13.2)
2	77 (35.2)
3	58 (26.5)
Age at menopause (years)	
<50	58 (26.5)
50–54	114 (52.1)
55	42 (19.2)
Missing	5 (2.3)
Lactation (months)	
No	105 (47.9)
0–12	62 (28.3)
>12	48 (21.9)
Missing	4 (1.8)
Body mass index (kg/m ²)	
<25	75 (34.2)
25–29	67 (30.6)
30	75 (34.2)
Missing	2 (1.0)
Weight gain in prior year (pounds)	
<–5	42 (19.2)
(–5) – 5	134 (61.2)
>5	32 (14.6)
Missing	1 (0.5)
Weight gain since age 18 (pounds)	
<0	15 (6.8)

	N (%)
0–20	40 (18.3)
21–50	89 (40.6)
>50	69 (31.5)
Missing	6 (2.7)
Alcohol consumption (drinks/wk)	
None	74 (33.8)
<5 per week	108 (49.3)
5 per week	29 (13.2)
Missing	8 (3.7)
Smoking history (pack-years)	
None	130 (59.4)
1–15	41 (18.7)
>15	39 (17.8)
Missing	9 (4.1)
Vigorous physical activity (h/wk)	
0–1.0	64 (29.2)
1.1–4.0	79 (36.1)
>4.0	76 (34.7)
Education	
High school	42 (19.2)
Some college	51 (23.3)
College diploma	64 (29.2)
Advanced degree	61 (27.9)
Missing	1 (0.5)

Table 2

Distribution of endogenous circulating molecules in study participants (N=219), Wisconsin Breast Density Study, 2008–2009.

	Median	Mean	SD	Correlation with Estradiol (P value)
Estradiol (pg/mL)	8.8	12.7	19.9	
Estrone (pg/mL)	26.0	30.7	17.6	0.85 (<0.0001)
Testosterone (ng/dL)	20.2	23.3	12.0	0.41 (<0.0001)
Sex hormone binding globulin (nmol/L)	42.9	45.8	23.3	−0.24 (0.0004)
Progesterone (pg/mL)	46.0	53.7	50.8	0.22 (0.0009)
IGF-1 (ng/mL) *	132.0	134.8	46.4	−0.22 (0.002)
Retinol (ng/mL) *	0.72	0.74	0.24	−0.20 (0.005)
Parathyroid hormone (pg/mL) *	36.7	41.1	21.1	0.18 (0.01)
25OHD (ng/mL) *	33.5	34.6	10.0	−0.13 (0.07)
Calcium (mg/dL) *	10.4	10.7	2.2	−0.10 (0.17)
IGFBP-3 (ug/mL) *	4.2	4.2	0.9	−0.04 (0.59)

* There were 15 subjects missing data on IGF-1, IGFBP-3, Calcium, 25OHD, Retinol and parathyroid hormone due to insufficient serum.

Table 3

Distribution of serum xenoestrogens and phytoestrogen in study participants (N=219), Wisconsin Breast Density Study, 2008–2009.

	Limit of Detection	No. (%) with detectable levels	Median Detected Value [†]	Maximum	Correlation with Estradiol (P value)
Nonylphenol (ng/mL)	0.06	88 (40.2)	15.4	725	0.23 (0.0005)
Coumestrol (ng/mL)	0.10	63 (28.8)	0.29	1.21	−0.12 (0.08)
Butyl paraben (ng/mL)	0.02	119 (54.3)	0.13	2.26	0.09 (0.16)
BPA (ng/mL)	0.24	54 (24.7)	0.81	14.5	−0.06 (0.34)
Propyl paraben (ng/mL) [*]	0.07	148 (67.6)	0.47	630	−0.04 (0.51)
Mono-ethyl phthalate (ng/mL)	0.11	32 (14.6)	4.93	130	−0.04 (0.60)
Genistein (ng/mL)	0.003	63 (28.8)	0.31	1.74	−0.03 (0.69)
Octylphenol (ng/mL)	0.25	29 (13.2)	1.88	58.2	0.02 (0.73)
Daidzein (ng/mL)	0.008	129 (58.9)	0.34	4.36	−0.0002 (0.99)

^{*}Data regarding serum propyl paraben was not available for 1 subject.

[†]Value displayed is the median of all samples with detectable values (i.e., excluding samples with non-detectable levels).

Table 4

The association between selected serum molecules and E-Screen activity (N=219), Wisconsin Breast Density Study, 2008–2009

Predictor	Linear regression [†]		Mean EEeq (ng/mL) ^{*,†} (95% Confidence Interval)				<i>P</i> _{trend}
	β coefficient	P	Q1	Q2	Q3	Q4	
Estradiol (pg/mL)	0.012	<.0001	0.024 (0.022, 0.027)	0.024 (0.022, 0.027)	0.029 (0.027, 0.032)	0.035 (0.032, 0.040)	<.0001
Estrone (pg/mL)	0.018	<.0001	0.021 (0.019, 0.022)	0.025 (0.023, 0.027)	0.031 (0.028, 0.033)	0.040 (0.036, 0.044)	<.0001
SHBG (nmol/L)	−0.004	<.0001	0.031 (0.029, 0.034)	0.029 (0.026, 0.031)	0.029 (0.027, 0.031)	0.024 (0.022, 0.026)	0.0004
IGFBP-3 (ug/mL)	0.147	<.0001	0.025 (0.022, 0.027)	0.029 (0.027, 0.031)	0.026 (0.024, 0.029)	0.033 (0.030, 0.036)	0.002
Testosterone (ng/dL)	0.004	0.02	0.025 (0.023, 0.027)	0.027 (0.025, 0.029)	0.030 (0.028, 0.033)	0.030 (0.028, 0.033)	0.0009
Progesterone (pg/mL)	−0.001	0.03	0.030 (0.027, 0.032)	0.029 (0.026, 0.031)	0.029 (0.027, 0.031)	0.026 (0.023, 0.028)	0.04
IGF-1 (ng/mL)	−0.001	0.08	0.029 (0.026, 0.032)	0.029 (0.027, 0.032)	0.027 (0.025, 0.029)	0.027 (0.025, 0.030)	0.34
Retinol (ng/mL)	−0.157	0.10	0.030 (0.027, 0.033)	0.028 (0.026, 0.030)	0.029 (0.027, 0.031)	0.026 (0.024, 0.028)	0.04

* Mean EEeq displayed is reverse transformed from model of log EEeq.

[†] Covariates include estradiol (continuous), estrone (continuous), SHBG (continuous), IGFBP-3 (continuous), testosterone (continuous), progesterone (continuous), IGF-1 (continuous), retinol (ng/mL), BMI (<25, 25–29, 30 kg/m²), alcohol consumption (0, <5, 5 drinks per week), and BPA (non-detectable, less than median detected value, median detected value).