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ORIGINAL REPORT

Inclusion of Endogenous Hormone Levels in Risk Prediction Models of Postmenopausal Breast Cancer

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose
Endogenous hormones are risk factors for postmenopausal breast cancer, and their measurement may improve our ability to identify high-risk women. Therefore, we evaluated whether inclusion of plasma estradiol, estrone, estrone sulfate, testosterone, dehydroepiandrosterone sulfate, prolactin, and sex hormone–binding globulin (SHBG) improved risk prediction for postmenopausal invasive breast cancer (n = 437 patient cases and n = 775 controls not using postmenopausal hormones) in the Nurses' Health Study.

Methods
We evaluated improvement in the area under the curve (AUC) for 5-year risk of invasive breast cancer by adding each hormone to the Gail and Rosner-Colditz risk scores. We used stepwise regression to identify the subset of hormones most associated with risk and assessed AUC improvement; we used 10-fold cross validation to assess model overfitting.

Results
Each hormone was associated with breast cancer risk (odds ratio doubling, 0.82 [SHBG] to 1.37 [estrone sulfate]). Individual hormones improved the AUC by 1.3 to 5.2 units relative to the Gail score and 0.3 to 2.9 for the Rosner-Colditz score. Estrone sulfate, testosterone, and prolactin were selected by stepwise regression and increased the AUC by 5.9 units ($P = .003$) for the Gail score and 3.4 ($P = .04$) for the Rosner-Colditz score. In cross validation, the average AUC change across the validation data sets was 6.0 ($P = .002$) and 3.0 units ($P = .03$), respectively. Similar results were observed for estrogen receptor–positive disease (selected hormones: estrone sulfate, testosterone, prolactin, and SHBG; change in AUC, 8.8 [$P < .001$] for Gail score and 5.8 [$P = .004$] for Rosner-Colditz score).

Conclusion
Our results support that endogenous hormones improve risk prediction for invasive breast cancer and could help identify women who may benefit from chemoprevention or more screening.

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INTRODUCTION

Breast cancer risk-prediction models have been developed to identify women at high risk who might benefit from increased frequency of screening, chemoprevention, or other risk-reduction strategies.[1] These models currently include reproductive history, family history of breast cancer, and other confirmed breast cancer risk factors,[2-4] but not endogenous hormone levels.

Substantial evidence supports a positive association of circulating estrogens, androgens, and prolactin with postmenopausal breast cancer risk.[5-12] In addition, having high levels of multiple hormones may further increase risk.[13] This suggests that including hormones may improve risk prediction and that multiple hormones may improve models the most. However, for cost effectiveness, it is important to minimize the number of hormones while maximizing improvement.

Although other factors, such as mammographic density and genetic markers, have been shown to improve risk prediction,[10,14-20] similar work incorporating hormones is lacking. Therefore, we assessed whether the inclusion of estradiol, estrone, estrone sulfate, testosterone, dehydroepiandrosterone sulfate (DHEAS), prolactin, and sex hormone–binding globulin (SHBG) improved risk prediction of postmenopausal invasive breast cancer. We considered two independently validated[21] risk scores: modified Gail[2,22] and Rosner-Colditz scores.[3,4,23]

METHODS

Study Population

We used data from the Nurses' Health Study, which was established in 1976 among 121,701 US female registered nurses age 30 to 55 years. Women completed a baseline questionnaire and have been observed biennially by questionnaire to update exposure status and disease diagnoses. From 1989 to 1990, 32,826 participants (age 43 to 69 years) provided blood samples.[24] From 2000 to 2002, 18,743 women provided a second blood sample (age 53 to 80 years).[12] Samples have been continuously stored in liquid nitrogen freezers. Follow-up of the blood cohort was 97% in 2010. This study was approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital (Boston, MA). Patient cases, who were postmenopausal and not using hormones (PMH) at blood draw, were diagnosed with invasive breast cancer after the initial blood collection but before June 1, 2010, and were matched to one or two controls on birth year (± 2 years), month (± 1 month) and time of day (± 2 hours) of blood draw, and fasting (< 8 *v* ≥ 8 hours).

Risk Scores

We estimated the 5- and 10-year risks of breast cancer using the Gail and Rosner-Colditz risk scores.[2-4,22] The Gail score includes: age at menarche, number of previous breast biopsies, presence of atypical hyperplasia at biopsy, age at first birth, number of first-degree relatives with a history of breast cancer, and age. We had information on ever or never having had a biopsy, so any woman reporting a biopsy was given a score of 1 for number of biopsies. We did not have complete data on atypical hyperplasia, so this term was excluded. Because the prevalence of atypical hyperplasia is low in the population, exclusion of this term does not substantially alter the calibration in our population.[25]

The Rosner-Colditz model includes: age at menarche, premenopausal duration (age at menopause minus age at menarche), postmenopausal duration (current age minus age at menopause), type of menopause, age at first birth minus age at menarche, birth index, history of benign breast disease, duration of PMH use by type (estrogen, estrogen plus progesterone, or other) and timing (current *v* past), body-mass index (BMI; trajectory from age 18 years to current), height, alcohol intake (from age 18 years to current), and family history of breast cancer. We included women not using PMH; therefore, we only considered past use.

Laboratory Assays

Hormone assay methods have been described previously.[9,12,26] Sex hormones were assayed by extraction/column chromatography (except DHEAS) followed by radioimmunoassay (RIA) or liquid chromatography–tandem mass spectrometry (LC-MS/MS) or chemiluminescent immunoassay (CEI [DHEAS]; Appendix, online only). In participant samples assayed by both methods (n = 10 to 21), the Pearson correlations ranged from 0.87 (95% CI, 0.54 to 0.97) for estrone to 0.98 (95% CI, 0.93 to 0.99) for testosterone (Appendix Table A1, online only). In general, mean levels between methods were similar, although LC-MS/MS had a higher mean for estrone sulfate than RIA, and for DHEAS, CEI had a lower mean than RIA. Prolactin was measured by microparticle enzyme immunoassay and SHBG by the AxSYM immunoassay system (Abbott Diagnostics, Abbott Park, IL).

Patient case–control sets and samples from the two blood collections were assayed together and labeled to mask patient case–control status. The coefficient of variation from blinded replicates was < 10% for 67% of batches and 10% to 15% otherwise. When hormone values were lower than the detection limit, we set the value at half this limit, and statistical outliers were excluded (Appendix, online only).[27,28] Mean hormone concentrations differed by batch; therefore, we adjusted levels as described in the Appendix (online only).[29,30]

We assayed samples from women who had not been using PMH for > 3 months before blood collection, because use alters hormone levels.[31] For patient cases diagnosed before June 1, 2000 (ie, before second collection), and their matched controls, we used hormone measures and risk score calculations assessed in 1990. For patient cases diagnosed from June 1, 2000, to May 31, 2010, and their matched controls, we used the prolactin value from the second blood draw, the average value from the two draws for the other hormones (if assay values from only one draw were available, we used data from that draw), and the risk score calculated in 2000. We used this approach because prolactin levels are only associated with risk for < 10 years after blood draw, whereas the other hormones predict risk for up to 20 years; thus, the average of the levels may best reflect exposure; secondary analyses only used values from the 2000 draw.[9,12] There were 525 potential patient cases available for analysis.

Statistical Analysis

We used unconditional logistic regression on $\log_2$-transformed hormone levels to assess relative risks (RRs) and 95% CIs with invasive breast cancer associated with a doubling in levels for each hormone separately and all hormones together, adjusting for matching factors. Adjustment for other risk factors did not substantially change the results. We used stepwise regression (entry $P < .15$ for forward and remain $P < .20$ for backward steps), adjusting for matching factors, to identify the subset of hormones that were most predictive of invasive breast cancer risk. To assess improvement in risk prediction, we compared the area under the curve (AUC), adjusting for age,[32] from a model only including a term for either the Gail or Rosner-Colditz risk score with that from a model with the risk score and a linear term for each $\log_2$ hormone.[33] We considered the AUC including the risk score with all hormones simultaneously and with the subset of hormones selected by stepwise regression. We secondarily considered use of quartiles for the hormones, with category cut points based on control distributions. We also examined the RR for an increase of one quartile in the predicted risk of breast cancer including the hormones and risk score, adjusting for quartiles of predicted risk for a model with just the risk score to assess model improvement.[34]

Secondarily, we conducted these analyses considering estrogen receptor (ER) –positive patient cases. We also excluded estrone sulfate from the stepwise regression, because this assay may not be commonly available clinically. We considered improvement in the AUC after adjusting for BMI, a routinely measured clinical variable that is correlated with estrogen levels.

To assess model overfitting, we used a 10-fold cross validation. The data set was divided into 10 approximately equal bins. The models were evaluated 10 times, using nine bins to conduct the stepwise regression and the 10th bin to assess improvement of the AUC. We averaged the change in AUC for the 10th bin across the 10 analyses and used fixed-effects meta-analysis to estimate the significance of the average change in AUC.[35] All *P* values were two sided and considered statistically significant if < .05. Analyses were conducted using SAS software (version 9.1; SAS Institute, Cary, NC).

RESULTS

We had 437 patient cases and 775 controls with data on all eight hormones and risk scores for the Gail model (n = 391 patient cases and 704 controls for Rosner-Colditz model; missing BMI at age 18 years led to the majority of the loss). Patient cases had a later age at first birth and were less likely to be parous but more likely to have benign breast disease and a family history of breast cancer (Table 1). The 5-year risk probability for breast cancer, using either model, was slightly higher for patient cases than controls. As expected, the median levels of the hormones (except SHBG) were higher in patient cases than controls.

The association between each hormone and breast cancer risk was statistically significant (Table 2). Estrone sulfate had the largest RR, for a doubling of 1.37 (95% CI, 1.22 to 1.54). Comparable RRs for the other hormones ranged from 1.15 (DHEAS) to 1.33 (estrone). SHBG was associated with a lower risk (RR, 0.82). When all hormones were included in the model, only estrone sulfate and prolactin remained statistically significant. This was likely because of the relatively high correlations among hormones, although the hormones were not strongly correlated with the risk scores (Appendix Table A2, online only). Using stepwise regression, estrone sulfate (RR, 1.33), testosterone (RR, 1.15), and prolactin (RR, 1.22) were selected.

Table 1. Baseline Demographic and Clinical Characteristics of Patient Cases and Matched Controls in Nurses' Health Study

Characteristic	Patient Cases (n = 437) Mean	Patient Cases (n = 437) SD	Controls (n = 775) Mean	Controls (n = 775) SD
Age at blood draw, years*	63.6	6.3	63.1	5.9
BMI at blood draw, kg/m^2	27.0	5.2	26.3	4.9
BMI at age 18 years, kg/m^2	21.5	2.9	21.7	2.9
Age at menarche, years	12.6	1.4	12.7	1.5
Age at first birth, years	31.9	20.4	30.2	18.0
Age at menopause, years	50.3	3.7	49.9	4.0
Physical activity, Met-hrs/wk	15.3	15.7	16.8	19.9
Alcohol consumption, g/d	4.8	8.6	5.2	10.0
Parous, %	89		91	
Previous history of benign breast disease, %	38		32	
Family history of breast cancer, %	18		14	
Mean 5-year breast cancer risk score				
Gail	0.021		0.020	
Rosner-Colditz†	0.021		0.019	

Hormone	Patient Cases (n = 437) Median	Patient Cases (n = 437) 10th to 90th Percentile	Controls (n = 775) Median	Controls (n = 775) 10th to 90th Percentile
Estrone, pg/mL	29.5	15.8 to 53.8	25.3	14.3 to 46.9
Estradiol, pg/mL	7.0	3.6 to 16.0	6.0	3.0 to 14.0
Estrone sulfate, pg/mL	268	110 to 706	210	96 to 520
Testosterone, ng/dL	22.0	12 to 38.3	20.0	11.4 to 37.0
DHEAS, μg/dL	61.0	26.6 to 153.1	58.5	21.9 to 129.9
SHBG, nmol/L	53.7	26.4 to 99.9	57.7	27.1 to 104.7
Prolactin, ng/mL	9.8	5.5 to 17.3	8.6	5.2 to 17.1

NOTE. Based on 1990 blood draw for patient cases diagnosed from 1990 to 2000 and matched controls and on 2000 blood draw for patient cases diagnosed from 2000 to 2010 and matched controls.
Abbreviations: BMI, body-mass index; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone–binding globulin; SD, standard deviation.
*Matching factor.
†There were 391 patient cases and 704 controls for Rosner-Colditz score.

Adding individual hormones to a model with either the Gail or Rosner-Colditz risk score improved the AUC for invasive breast cancer (Table 3). The AUC for the Gail score alone was 54.9; for the Rosner-Colditz score alone, it was 58.9. For the Gail score, adding estrone or estrone sulfate significantly improved the AUC (by 3.7 and 5.2 units, respectively). For the Rosner-Colditz score, no individual hormone improved the AUC significantly, although estrone sulfate changed the AUC by 2.9 units ($P = .06$). Inclusion of all hormones in the model significantly increased the AUC by 5.9 units for the Gail score ($P = .003$) and 3.3 units for the Rosner-Colditz score ($P = .04$). Inclusion of estrone sulfate, prolactin, and testosterone led to the same improvement in AUC (5.9 and 3.4 units, respectively). After adjusting for predicted risk including either score alone, the RR for a one-quartile increase in the predicted risk when adding estrone sulfate, prolactin, and testosterone was 1.3 ($P < .001$) for the Gail score and 1.4 ($P < .001$) for the Rosner-Colditz score (Appendix Table A3, online only). Results were similar when using the 10-year risk score (data not shown). The average improvement in the AUC in 10-fold cross validation was 6.0 units ($P = .002$) with the Gail score and 3.0 units ($P = .03$) with the Rosner-Colditz score.

For ER-positive tumors, the AUC for the Gail score alone was 54.4, and for the Rosner-Colditz score, it was 59.1 (Table 4). The improvement when individual hormones were added to the model ranged from 2.7 to 6.4 for the Gail score and 1.0 to 3.7 units for the Rosner-Colditz score; including all hormones led to a 9.1- ($P < .001$) and 6.0-unit ($P = .003$) increase in the AUC, respectively. In stepwise regression, estrone sulfate, testosterone, prolactin, and SHBG were selected. Inclusion of these hormones improved the AUC for the Gail score by 8.8 ($P < .001$) and for the Rosner-Colditz score by 5.8 units ($P = .004$). After adjusting for predicted risk of ER-positive disease using either score alone, the RR for a one-quartile increase in the predicted risk when adding estrone sulfate, prolactin, SHBG, and testosterone was 1.5 ($P < .001$) for the Gail score and 1.6 ($P < .001$) for Rosner-Colditz score (Appendix Table A4, online only). The average improvement in the AUC in cross validation was 7.3 units ($P = .001$) for the Gail score and 4.5 units ($P = .01$) for the Rosner-Colditz score.

When removing estrone sulfate from stepwise regression, we selected estrone, testosterone, SHBG, and prolactin for invasive breast cancer and estradiol, testosterone, SHBG, and prolactin for ER-positive disease. The improvement in the AUC for invasive breast cancer including these hormones was 4.9 units ($P = .01$) for the Gail score and 2.4 ($P = .13$) for the Rosner-Colditz score; for ER-positive disease, the corresponding change was 8.4 ($P < .001$) and 4.9 units

Table 2. RR of Invasive Breast cancer for Doubling of Hormone Levels

Hormone	Hormones Included Individually RR	Hormones Included Individually 95% CI	Hormones Included Simultaneously RR	Hormones Included Simultaneously 95% CI	Hormones Selected Using Stepwise Regression RR	Hormones Selected Using Stepwise Regression 95% CI
Estrone	1.33	1.14 to 1.54	1.04	0.84 to 1.29	—	—
Estradiol	1.30	1.13 to 1.49	0.99	0.80 to 1.23	—	—
Estrone sulfate	1.37	1.22 to 1.54	1.28	1.08 to 1.51	1.33	1.17 to 1.51
Testosterone	1.29	1.09 to 1.53	1.16	0.94 to 1.43	1.15	0.96 to 1.37
DHEAS	1.15	1.04 to 1.29	0.99	0.87 to 1.13	—	—
SHBG	0.82	0.70 to 0.95	0.90	0.76 to 1.08	—	—
Prolactin	1.24	1.04 to 1.47	1.22	1.02 to 1.46	1.22	1.02 to 1.46

NOTE. Multivariable models adjusted for fasting status (< 8 *v* ≥ 8 hours), time of day (24-hour clock: < 8*v* 8 to 12 *v* 13 to 24), age at blood draw (continuous), and season of blood draw (May to October *v* other month). Entry $P < .15$ for forward steps and remain $P < .20$ for backward steps in stepwise regression models.
Abbreviations: DHEAS, dehydroepiandrosterone sulfate; RR, relative risk; SHBG, sex hormone–binding globulin.

Table 3. Change in Age-Adjusted AUC for Invasive Breast Cancer

Risk Score	Gail Score*					Rosner-Colditz Score†				
	AUC	SE	Change in AUC	SE	*P*‡	AUC	SE	Change in AUC	SE	*P*‡
Alone	54.9	1.7	—	—	—	58.9	1.8	—	—	—
Plus estrone	58.6	1.7	3.7	1.7	.03	60.6	1.8	1.8	1.3	.18
Plus estradiol	57.7	1.7	2.9	1.7	.10	59.6	1.8	0.7	1.3	.57
Plus estrone sulfate	60.1	1.7	5.2	1.9	.007	61.8	1.8	2.9	1.6	.06
Plus testosterone	57.1	1.7	2.3	1.7	.18	59.9	1.8	1.0	1.2	.39
Plus DHEAS	57.5	1.7	2.7	1.5	.08	60.4	1.8	1.6	1.2	.18
Plus SHBG	56.2	1.7	1.3	1.6	.41	59.1	1.8	0.3	1.0	.79
Plus prolactin	57.3	1.7	2.5	1.6	.12	59.8	1.8	1.0	1.2	.42
All hormones	60.8	1.7	5.9	2.0	.003	62.2	1.8	3.3	1.7	.04
Estrone sulfate, testosterone, and prolactin§	60.8	1.7	5.9	2.0	.003	62.3	1.8	3.4	1.7	.04
Using quartile categories for above three hormones	61.6	1.7	6.7	2.0	.001	63.0	1.7	4.1	1.7	.02

NOTE. Adding endogenous hormone levels to Gail or Rosner-Colditz 5-year risk score.
Abbreviations: AUC, area under the curve; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone–binding globulin.
*Patient cases, n = 437; controls, n = 775.
†Patient cases, n = 391; controls, n = 704.
‡*P* value indicates difference in AUC from model with only risk score.
§Hormones selected via stepwise logistic regression; beta coefficients for best-subset hormones were 0.304 for estrone sulfate, 0.153 for testosterone, and 0.200 for prolactin for Gail score and 0.266, 0.156, and 0.232, respectively, for Rosner-Colditz score.

($P = .01$), respectively. The increase in the AUC was slightly attenuated after BMI adjustment, although adding estrone sulfate, testosterone, and prolactin still led to increases in the AUC (4.6 units for Gail score and 3.0 for Rosner-Colditz score). Results were similar when excluding individuals with hormone levels lower than the limit of detection or when using only a single measure of the sex hormones (data not shown) and were similar or slightly stronger when using quartile categories of the hormone levels rather than continuous variables (Tables 3 and 4).

DISCUSSION

In this large prospective study, we observed that inclusion of endogenous hormones modestly improved breast cancer risk prediction among postmenopausal women not using PMH. Results from 10-fold cross validation suggested little to no overfitting. Adding three hormones—estrone sulfate, testosterone, and prolactin—provided the largest improvement in prediction with the fewest hormones for invasive breast cancer; the best subset for ER-positive disease also included SHBG. These biomarkers represent three modestly correlated hormonal axes—estrogens, androgens, and growth hormones—suggesting that consideration of the broad hormonal milieu may provide the most information with respect to risk prediction.

Our results are consistent with those of other studies of these hormones and breast cancer risk.[5-9,11,12] Estrone sulfate improved prediction more than other hormones and was selected in stepwise regression models. This may be because, at least in our data, estrone sulfate was more strongly associated with ER-negative disease than the

Table 4. Change in Age-Adjusted AUC for ER-Positive Breast Cancer

Risk Score	Gail Score*					Rosner-Colditz Score†				
	AUC	SE	Change in AUC	SE	*P*‡	AUC	SE	Change in AUC	SE	*P*‡
Alone	54.4	1.9	—	—	—	59.1	2.0	—	—	—
Plus estrone	60.2	1.9	5.8	2.0	.005	62.3	2.0	3.2	1.8	.07
Plus estradiol	59.5	1.9	5.1	2.1	.01	61.2	2.0	2.1	1.7	.22
Plus estrone sulfate	60.8	1.9	6.4	2.2	.003	62.8	1.9	3.7	1.8	.04
Plus testosterone	58.5	1.9	4.2	2.0	.04	61.1	2.0	2.0	1.6	.20
Plus DHEAS	58.2	1.9	3.8	1.7	.03	61.1	2.0	2.0	1.4	.14
Plus SHBG	57.1	1.9	2.7	1.8	.13	60.1	2.0	1.0	1.4	.41
Plus prolactin	59.5	1.9	5.1	2.0	.01	62.0	2.0	2.9	1.7	.08
All hormones	63.5	1.8	9.1	2.3	< .001	65.1	1.9	6.0	2.0	.003
Estrone sulfate, SHBG, testosterone, and prolactin§	63.2	1.8	8.8	2.3	< .001	64.9	1.9	5.8	2.0	.004
Using quartile categories for above four hormones	63.8	1.8	9.4	2.3	< .001	65.5	1.9	6.4	2.0	.002

NOTE. Adding endogenous hormone levels to Gail or Rosner-Colditz 5-year risk score.
Abbreviations: AUC, area under the curve; DHEAS, dehydroepiandrosterone sulfate; ER, estrogen receptor; SHBG, sex hormone–binding globulin.
*Patient cases, n = 321.
†Patient cases, n = 283.
‡*P* value indicates difference in AUC from model with only risk score.
§Hormones selected via stepwise logistic regression; beta coefficients for best-subset hormones were 0.280 for estrone sulfate, 0.236 for testosterone, −0.197 for SHBG, and 0.336 for prolactin for Gail score and 0.256, 0.237, −0.150, and 0.370, respectively, for Rosner-Colditz score.

other estrogens (eg, estrone sulfate RR doubling, 1.43 for ER-positive and 1.40 for ER-negative disease; estradiol RR, 1.41 for ER-positive and 0.93 for ER-negative disease); thus, it was a stronger predictor of overall risk. Baglietto et al[5] reported similar associations for estrone sulfate by ER status, although analogous associations were observed for estradiol and estrone. Furthermore, estrone sulfate has a wider distribution than the other estrogens and can act as a reservoir for bioactive estrogens; therefore, it may provide more information on overall estrogen status. Because estrone sulfate assays are less common clinically, we conducted the analysis excluding this hormone. Under these conditions, estrone, testosterone, SHBG, and prolactin were chosen; the improvements in the AUC were approximately 1 unit lower.

We also considered ER-positive disease, because these hormones are more strongly associated with this subtype.[9,12] The AUC improved more for ER-positive disease than for total invasive breast cancer, suggesting that development of a risk model for ER-positive disease may lead to better predictive ability and more targeted use of chemoprevention that prevents ER-positive tumors.[36,37] It is possible that we overestimated the improvement, because factors such as BMI, which are more strongly associated with ER-positive disease and correlated with hormone levels, were not weighted as heavily in the risk score. One study examined hormones in a risk prediction model of postmenopausal ER-positive disease,[10] considering risk factors, single-nucleotide polymorphisms, estradiol, testosterone, prolactin, insulin-like growth factor 1 (IGF-1), and IGF binding protein-3. The final model included age, BMI, age at menopause, *TP53* single-nucleotide polymorphism, testosterone, and IGF-1 and had an AUC of 77.0. No comparison model was used, nor was overfitting assessed. Because the hormones selected in that study were different than those selected in ours, it will be important to consider whether there are racial/ethnic differences in which hormones may best improve prediction.

Biologically, estrogens, androgens, and prolactin likely have independent and synergistic mechanisms of action regarding breast carcinogenesis. For example, administration of testosterone and estradiol together, but not alone, led to the development of invasive mammary tumors in rats.[38,39] Likewise, in a breast cancer cell line that secreted high prolactin levels, estradiol magnified proliferation.[40] Most estrogens, androgens, and prolactin can either bind or regulate ER, but androgens may also act as a reservoir for estrogen synthesis or through the androgen receptor; prolactin may act through the prolactin receptor.[41-44]

The observed improvement in the AUC when adding hormone levels was similar to prior studies that added genetic markers or mammographic density.[14-16,18,20] For example, genetic factors increased the AUC by 3 to 7 units versus the Gail model alone,[14-16,20] and mammographic density increased the AUC by 4.7 units.[18] Hormones are not strongly correlated with genetic factors or mammographic density, and in prior studies, they have been shown to have independent associations with breast cancer risk and potentially additive effects.[45-47] Thus, future studies should consider simultaneous inclusion of these factors as well as other lifestyle factors.[48,49]

In our nested case-control study, the Gail and Rosner-Colditz scores had slightly lower discriminatory value than in the full cohort (AUC for full cohort *v* current study: Gail, 58 *v* 55; Rosner-Colditz, 63 *v* 57).[3,25] This may be because patient cases and controls were matched on age, which contributed to both scores. However, because age is a strong risk factor, it may be most useful to discriminate among women of the same age. Current PMH use contributes to the Rosner-Colditz score, and not having current users may have lowered the predictive value within our population. Furthermore, we only included postmenopausal women not using hormones, although the importance of endogenous hormones in breast cancer risk is most marked in this group, and therefore, they are often targeted for breast cancer prevention. We also used a modified Gail model, because we did not have information on atypical hyperplasia, which is rare, altering the scores for only a few women. In addition, we used risk scores in the model rather than the individual factors because of the sample size. If hormones are strongly correlated with factors in the model, we may have slightly overestimated improvement when adding hormones. However, BMI is the primary factor (in Rosner-Colditz score) associated with these hormones, namely estrogens, and we still observed large increases in the AUC after adjusting for BMI. Finally, we used different sex hormone assays over time, reflecting the best assay technology available when the samples were assayed. This may have led to some measurement error and precluded us from reporting on relationships with absolute hormone concentrations. Strengths include that blood samples were collected years before disease onset, and we assayed many hormones, allowing for a detailed evaluation of the best subset of hormones for risk prediction. We had 20 years of follow-up and blood samples drawn at two time points, allowing us to maximize our sample size.

Inclusion of endogenous hormones, particularly estrone sulfate, testosterone, and prolactin, modestly improved discrimination of both total invasive and ER-positive breast cancers in postmenopausal women not using PMH. The influence of hormones on predicting absolute risk and assessment of calibration is needed to evaluate clinical utility. Finally, a cost-benefit analysis weighing the assay costs against additional predictive value is critical,[50] although our results suggest that these hormones would only need to be measured every 10 years. Importantly, the Centers for Disease Control and Prevention is developing a standardization program for sex hormone assays,[51,52] which is essential for clinical implementation. If our results are confirmed, inclusion of hormones in risk prediction could lead to more targeted chemoprevention and screening in high-risk women.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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GLOSSARY TERMS

androgen receptor: a DNA-binding and hormone-activated transcription factor important to the development and progression of prostate cancer. Its primary ligand is dihydrotestosterone. In later-stage (castration-resistant) prostate cancer, oncogenic alterations such as androgen receptor overexpression allow the androgen receptor to continue signaling despite undetectable, or castrate, levels of serum testosterone.

estrogen receptor (ER): ligand-activated nuclear proteins, belonging to the class of nuclear receptors, present in many breast cancer cells that are important in the progression of hormone-dependent cancers. After binding, the receptor-ligand complex activates gene transcription. There are two types of estrogen receptors (ERα and ERβ). ERα is one of the most important proteins controlling breast cancer function. ERβ is present in much lower levels in breast cancer, and its function is uncertain. Estrogen receptor status guides therapeutic decisions in breast cancer.

risk score: a simplified version of a prognostic model, in which scores are assigned to each risk factor (eg, on the basis of rounded regression coefficients).

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Appendix

Estrone, estradiol, testosterone, and dehydroepiandrosterone sulfate (DHEAS) were assayed at Quest Diagnostics (San Juan Capistrano, CA) using an extraction step and column chromatography (except DHEAS) followed by radioimmunoassay (RIA) for patient cases diagnosed from 1990 to 2000 and matched controls; subsequent samples were assayed at the Mayo Clinic (Rochester, MN) by liquid chromatography–tandem mass spectrometry (LC-MS/MS; estradiol, estrone, and testosterone) or solid-phase competitive chemiluminescent enzyme immunoassay (DHEAS). For estrone sulfate, patient cases and controls identified through 2004 were assayed at the University of Massachusetts Medical Center Longcope Radioimmunoassay Laboratory (Amherst, MA) or at Quest Diagnostics by extracting estrone sulfate, enzymatically cleaving the sulfate bond to release estrone, extracting the released estrone by chromatography, and conducting RIA. The remaining samples were assayed at the Mayo Clinic by LC-MS/MS. Sex hormone–binding globulin (SHBG) and prolactin were assayed at Longcope or the Massachusetts General Hospital Reproductive Endocrinology Unit Laboratory (Boston, MA). Prolactin was measured by microparticle enzyme immunoassay and SHBG by the AxSYM immunoassay system (Abbott Diagnostics, Abbott Park, IL).

In participant samples assayed by both methods for the sex hormones (n = 10 to 21), the Pearson correlations ranged from 0.87 (95% CI, 0.54 to 0.97) for estrone to 0.98 (95% CI, 0.93 to 0.99) for testosterone (Appendix Table A1). Spearman correlation coefficients were similar (all > 0.90). In general, mean levels among methods were similar, although LC-MS/MS led to higher mean levels for estrone sulfate than RIA, and for DHEAS, chemiluminescent immunoassay resulted in lower mean levels than RIA. Limits of detection and number of samples below the limit were: 2 pg/mL for estradiol (n = 0), 10 pg/mL for estrone (n = 0), 40 pg/mL for estrone sulfate (n = 7), 2 ng/dL for testosterone (n = 0), 5 ug/dL for DHEAS (n = 2), 0.6 ng/mL for prolactin (n = 0), and 2 nmol/L for SHBG (n = 0). We excluded statistical outliers for each hormone (ranging from 0 [DHEAS] to nine [estradiol]).

Because of batch-to-batch variation resulting in part from using different assay modalities over time, we used a statistical technique to recalibrate each assay batch to an average batch for all hormones except testosterone. We assumed that all batches combined represented an average batch. We then regressed levels of each sex hormone on age, body-mass index, patient case or control status, and other factors related to the hormones that may have varied by batch as well as indicator variables for each batch. Within each batch, sex hormone levels were recalibrated by adding the resulting value of the coefficients for that batch minus the average of the batch coefficients. Therefore, these recalibrated levels accounted for the variability between batches independent of varying covariate distributions between batches. For testosterone, we had samples run in each batch over time, and we recalibrated values from all batches to have a comparable distribution to the final batch. To do this, we used linear regression, separately by batch, to assess the relationship between the assay value measured in the final batch and that measured in the original batch and used the intercept and beta coefficient to rescale all of the values in the original batch.

Table A1. Relationship Between Different Assay Modalities for Sex Hormones Measured in Nurses' Health Study

Hormone	Original		New		No. of Participants	Spearman Correlation	*P*	Pearson's Correlation	95% CI	Mean	
	Method	Follow-Up (years)	Method	Follow-Up (years)						Original	New
Estrone	Ext RIA	1990 to 2000	LC-MS/MS	2000 to 2010	10	0.92	< .001	0.87	0.54 to 0.97	26.5	29.5
Estradiol	Ext RIA	1990 to 2000	LC-MS/MS	2000 to 2010	10	0.90	< .001	0.96	0.85 to 0.99	8.6	7.1
Estrone sulfate	Ext RIA	1990 to 2004	LC-MS/MS	2004 to 2010	17	0.96	< .001	0.98	0.93 to 0.99	1,322	1,900
Testosterone	Ext RIA	1990 to 2000	LC-MS/MS	2000 to 2010	21	0.95	< .001	0.96	0.90 to 0.98	21.0	21.8
DHEAS	RIA	1990 to 2000	CEI	2000 to 2010	12	0.95	< .001	0.98	0.92 to 0.99	177	108

NOTE. With follow-up from 1990 to 2010.
Abbreviations: CEI, chemiluminescent enzyme immunoassay; DHEAS, dehydroepiandrosterone sulfate; Ext, extraction; LC-MS/MS, liquid chromatography–mass spectrometry; RIA, radioimmunoassay.

Table A2. Spearman Correlations Among Sex Hormones and Prolactin in Postmenopausal Controls Not Using PMH (n = 775)

Hormones	Estradiol	Estrone	Estrone sulfate	Testosterone	DHEAS	SHBG	Prolactin	Age	Gail Score	Rosner-Colditz Score
Estradiol	1.00	0.77	0.57	0.40	0.31	−0.36	−0.07*	−0.08	−0.05*	0.10
Estrone		1.00	0.65	0.49	0.42	−0.22	0.02*	−0.02*	−0.05*	0.07*
Estrone sulfate			1.00	0.28	0.49	−0.36	−0.02*	−0.08	−0.07*	0.05*
Testosterone				1.00	0.41	0.09	0.11	0.08	−0.02*	0.13
DHEAS					1.00	−0.09	0.05*	−0.20	−0.14	−0.002*
SHBG						1.00	0.04*	0.03*	0.05*	−0.08*
Prolactin							1.00	0.02*	−0.03*	0.06*
Age								1.00	0.44	0.39
Gail score									1.00	0.53
Rosner-Colditz score										1.00

NOTE. All correlations were statistically significant at $P < .05$ except where otherwise noted.
Abbreviations: DHEAS, dehydroepiandrosterone sulfate; PMH, postmenopausal hormone; SHBG, sex hormone–binding globulin.
*Not statistically significant.

Table A3. Patient Cases and Controls by Quartile of Predicted Risk of Invasive Breast Cancer When Including Estrone Sulfate, Prolactin, and Testosterone

Risk Score Alone	Quartile One		Quartile Two		Quartile Three		Quartile Four		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Gail Score										
Quartile one										
Patient cases	21	23	22	24	23	25	25	27	91	100
Controls	61	31	51	26	46	24	36	19	194	100
Quartile two										
Patient cases	17	18	28	29	25	26	26	27	96	100
Controls	59	31	48	25	49	25	37	19	193	100
Quartile three										
Patient cases	15	15	18	19	29	30	35	36	97	100
Controls	57	29	56	29	47	24	34	18	194	100
Quartile four										
Patient cases	12	8	27	18	32	21	82	54	153	100
Controls	16	8	39	20	52	27	87	45	194	100
Rosner-Colditz Score										
Quartile one										
Patient cases	24	39	21	34	12	20	4	7	61	100
Controls	88	50	47	27	27	15	14	8	176	100
Quartile two										
Patient cases	15	17	22	26	24	28	25	29	86	100
Controls	55	31	54	31	46	26	21	12	176	100
Quartile three										
Patient cases	9	8	25	22	37	33	42	37	113	100
Controls	28	16	51	29	57	32	40	23	176	100
Quartile four										
Patient cases	2	2	11	8	37	28	81	62	131	100
Controls	5	3	24	14	46	26	101	57	176	100

NOTE. Quartiles when adding estrone sulfate, prolactin, and testosterone stratified by quartiles when including Gail or Rosner-Colditz score alone. Odds ratio for one-unit increase in quartile of predicted risk when including hormones (adjusting for quartiles of predicted risk for risk score only) was 1.3 ($P < .001$) for Gail score and 1.4 ($P < .001$) for Rosner-Colditz score.

Table A4. Patient Cases and Controls by Quartile of Predicted Risk of ER-Positive Breast Cancer When Including Estrone Sulfate, Prolactin, Testosterone, and SHBG

Risk Score Only	Quartile One		Quartile Two		Quartile Three		Quartile Four		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Gail Score										
Quartile one										
Patient cases	18	25	11	15	14	19	29	40	72	100
Controls	66	34	46	24	34	18	47	24	193	100
Quartile two										
Patient cases	10	14	14	19	19	26	29	40	72	100
Controls	56	29	49	25	49	25	40	21	194	100
Quartile three										
Patient cases	5	7	11	15	30	42	22	31	68	94
Controls	48	25	58	30	48	25	41	21	195	101
Quartile four										
Patient cases	8	11	20	28	26	36	55	76	109	151
Controls	23	12	42	22	62	32	66	34	193	100
Rosner-Colditz Score										
Quartile one										
Patient cases	16	35	11	24	11	24	8	17	46	100
Controls	86	49	43	24	28	16	19	11	176	100
Quartile two										
Patient cases	9	15	10	17	20	33	21	35	60	100
Controls	53	30	47	27	48	27	28	16	176	100
Quartile three										
Patient cases	6	8	10	13	33	42	30	38	79	100
Controls	29	16	60	34	47	27	40	23	176	100
Quartile four										
Patient cases	2	2	5	5	33	34	58	59	98	100
Controls	8	5	26	15	53	30	89	51	176	100

NOTE. Quartiles when adding estrone sulfate, prolactin, SHBG, and testosterone stratified by quartiles when including Gail or Rosner-Colditz score alone. Odds ratio for one-unit increase in quartile of predicted risk when including hormones (adjusting for quartiles of predicted risk for risk score only) was 1.5 ($P < .001$) for Gail score and 1.6 ($P < .001$) for Rosner-Colditz score.

Abbreviations: ER, estrogen receptor; SHBG, sex hormone–binding globulin.