Published in final edited form as:
Breast Cancer Res Treat. 2013 February ; 137(3): 883–892. doi:10.1007/s10549-012-2391-z.

Postmenopausal plasma sex hormone levels and breast cancer risk over 20 years of follow-up

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Abstract

Background—Plasma estrogen and androgen levels are positively associated with postmenopausal breast cancer risk, but how long a single blood measurement can predict risk and whether the associations vary by tumor hormone receptor status remain unclear.

Methods—We conducted nested case-control analyses within the Nurses' Health Study. Blood samples were collected in 1989-1990 and again in 2000-2002. Among postmenopausal women not using postmenopausal hormones at blood collection, 707 cases were diagnosed through June 2010, with two matched controls per case. We used unconditional logistic regression analyses to estimate the relative risks controlling for other breast cancer risk factors.

Results—The intra-class correlation coefficients for two blood measurements collected 10 years apart ranged from 0.54 (dehydroepiandrosterone sulfate, DHEAS) to 0.74 (sex hormone-binding globulin, SHBG). Overall, women in the top (vs. bottom) 25% of levels of estradiol, free estradiol, testosterone, free testosterone, and DHEAS were at a 50-110% higher risk of breast cancer (p_{trend}<0.001). SHBG was inversely associated with risk (p_{trend}=0.004). RRs were similar when comparing cases diagnosed 1-10 vs. 11-20 years (or 16-20years) after blood collection ($p_{interaction}$>0.2). Except for DHEAS, the associations varied significantly by hormone receptor status ($p_{heterogeneity}$≤0.02). For example, the RRs (95% CIs) comparing the highest vs. lowest quartile were 2.8 (2.0-4.0; p_{trend}<0.001) for ER+/PR+ tumors vs. 1.1 (0.6-2.1; p_{trend}=0.98) for ER-/PR- tumors for estradiol, and 1.8 (1.3-2.5; p_{trend}<0.001) vs. 0.6 (0.3-1.2; p_{trend}=0.35) for testosterone.

Conclusions—One measure of circulating sex hormones in postmenopausal women can predict risk of hormone receptor positive breast cancer for up to 16-20 years.

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The results presented in this manuscript have not been considered for publication elsewhere. We presented a poster showing preliminary results of this manuscript in 2012 AACR Frontiers meeting.

Ethical standards: The experiments comply with the current laws of the country in which they were performed.

Conflict of interest: The authors declare that they have no conflict of interest. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Introduction

Despite the well-established associations between sex hormone levels and postmenopausal breast cancer risk [1, 6, 20, 21, 26, 29], it is unknown how long a single blood hormone measurement can predict subsequent breast cancer risk because most prior studies have included less than 10 years of follow-up [29]. Including hormone levels in breast cancer risk prediction models [19, 37, 38] might improve discriminative ability and thus better identify high-risk women who would benefit most from heightened screening or chemoprevention. Therefore, evaluating timing can help determine the most appropriate frequency for hormone measurements. Further, breast cancer is a heterogeneous disease [2, 10, 42] and it remains uncertain to what extent the hormone-breast cancer associations vary by tumor subtypes such as those defined by estrogen receptor (ER) and progesterone receptor (PR) status. Few studies have examined this potential difference and results have been mixed [3, 11, 16, 25, 27, 33, 40, 46].

Previously, we reported statistically significant associations between pre-diagnostic sex hormone levels and postmenopausal breast cancer risk, including 322 cases over 8 years of follow-up [33]. The current analysis now includes 20 years of follow-up and an additional 400 cases.

Materials and Methods

Study Population

The Nurses' Health Study (NHS) and the blood collection have been described in detail [5, 9, 21, 22, 33]. Briefly, during 1989-1990, an initial blood sample was collected from 32,826 cohort members, who were 43-69 years of age. Each woman arranged to have her blood drawn and shipped, via overnight courier with an ice pack, to our laboratory, where it was processed. A second blood sample was collected from a subset of these women in 2000-2002; 25,947 women who were still alive, had responded to a recent questionnaire, and had no history of cancer, were invited to participate. Among these, 21,600 (83%) agreed, 2185 (8%) declined, and the remainder did not reply. Samples were collected using a similar protocol to that used in the first collection. Overall, 18,743 women (ages 53-80 years, >98% postmenopausal) returned samples to our laboratory (72% of invited). As of 2010, the follow-up rate among the women who provided blood samples was 97.2%.

The breast cancer cases and controls in this analysis are women who, at either blood collection, were postmenopausal and had not used postmenopausal hormones for at least 3 months. Cases who did not provide a second blood sample or were diagnosed prior to the 2nd collection, contributed one blood sample to the analysis; cases diagnosed after donating a second blood sample provided one or two samples to the analysis, depending on their menopausal and hormone use status at each draw.

All breast cancer cases were confirmed by medical records or verbally by the nurse [15]. We matched two control subjects (except for DHEAS, 1:1 match for 2002-2008 cycles for financial reasons) per case by age (±1 year), month (±1 month) and time of day (±2 hours) of blood collection, and fasting status (≥10 hours since a meal vs. <10 hours or unknown) at each blood collection. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital, Boston, Massachusetts.

Laboratory Analysis

Hormone assay methods for the follow-up years from 1990 to 1998 have been described previously [21, 33]. In brief, estradiol, DHEAS, and testosterone were measured at either Quest Diagnostics (San Juan Capistrano, CA) by radioimmunoassay (from 1990 to 1998) or

the Mayo Clinic (Rochester, MN) by liquid chromatography-tandem mass spectrometry (for estradiol and testosterone) or by a solid-phase, competitive chemiluminescent enzyme immunoassay (for DHEAS; Siemens Healthcare Diagnostics, Deerfield, IL) for recent follow-up years (i.e., 2000-2010). The correlation was >0.9 between these two assay methods. The first 2 of 6 batches of SHBG were assayed at the Longcope Laboratory; and all other batches of SHBG were assayed at Massachusetts General Hospital (Boston MA) using a solid-phase two-site chemiluminescent enzyme immunometric assay (Immulite; DPC, Inc). Free estradiol and free testosterone were calculated by the law of mass action [41]. Laboratory technicians were blinded to case-control status and matched case-control sets were assayed together; similarly both bloods from a participant were assayed together in the same batch blinding the laboratory to which samples belonged to the same individual. Within-batch laboratory coefficients of variations ranged from 8%-12%.

Assessment of Other Covariates

We obtained information on breast cancer risk factors such as age at menarche, height, weight at age 18, current weight, age at first birth, parity, and family history of breast cancer, diagnosis of benign breast disease, and age at menopause at baseline or on subsequent questionnaires.

Statistical Analysis

We categorized plasma hormone levels into quartiles using cut points based on the control distribution. We used the cut-offs from the first blood sample because hormone distributions were comparable across blood draws. We used the lowest quartile as the reference group for all analyses and calculated tests for trend by modeling the quartile medians. All p-values were two-sided with $p<0.05$ indicating significance. For estradiol, the mean and standard deviation of both the control values and the quality-control replicates were very similar across batches; thus, quartile cut points were made according to the distribution in the control subjects overall. For testosterone, the median values from the 1994 and 1996 batches were higher than those of all other batches. Using 8-10 participant samples measured in 1994 or 1996 and re-tested in the 1998 batch, we recalibrated these two batches to the 1998 batch using regression calibration [43]. For SHBG and DHEAS, because identical QC samples were not included across batches, we used average batch recalibration [39], taking into account batch and participant factors that may have varied by batch and are important predictors of SHBG (BMI) and DHEAS (age). Results using recalibrated data versus batch-specific cut-offs were comparable, hence only recalibrated data are presented here.

We identified outliers of each hormone [36]. We tested for differences in mean hormone levels between cases and controls using a mixed-effects regression model [30].

We used unconditional logistic regression analyses, controlling for matching factors and potential or established risk factors for breast cancer (see Table 2 footnote for details), to estimate relative risks (RRs). We used unconditional logistic regression to increase our statistical power in some analyses; conditional logistic regression models yielded similar results.

We first evaluated whether hormone levels measured <10 or >10-20 (and also 16-20) years before breast cancer diagnosis were associated with breast cancer risk. We tested whether the associations with each hormone differed by follow-up period by creating a cross-product term between hormone (medians of categories) and follow-up period, and evaluated whether the beta-coefficient of this product term was statistically significant using a Wald test.

Because no significant heterogeneity by follow-up period was observed (p-interaction>0.2), we combined all data to maximize statistical power to assess hormone-breast cancer

associations by breast cancer subtypes. In this analysis, among women with both bloods, we used the average values over time to best represent their long-term levels.

We additionally tested for differences by breast cancer tumor characteristics using polychotomous logistic regression [32] with three end points for tumor invasiveness (invasive, in situ, no breast cancer) and four end points for tumor receptor status (ER+/PR+, ER+/PR-, ER-/PR-, no breast cancer).

To evaluate the independent associations of each hormone, we conducted additional analyses entering two hormones at a time into the model. We evaluated if the hormone associations varied across strata of other factors, including age at blood collection (median=62 years), age at diagnosis (median=69 years), age at menopause (median=50 years), BMI at age 18 (median=26 kg/m^2), benign breast disease (yes/no), and family history of breast cancer (yes/no).

Results

Overall, 707 cases were diagnosed through June 2010. The mean age was 61 years at the first and 69 years at the second collection (Table 1). Compared with controls, cases were statistically significantly more likely to have benign breast disease or a family history of breast cancer. Circulating steroid hormone levels were statistically significantly higher among cases than controls for all hormones investigated, except SHBG, where higher levels were observed among controls.

The intra-class correlation coefficients for the two blood measures collected 10 years apart were 0.69 (95%CI: 0.61-0.75) for estradiol, 0.71 (95%CI: 0.67-0.75) for testosterone, 0.54 (95%CI: 0.45-0.62) for DHEAS, and 0.74 (95%CI: 0.67-0.80) for SHBG.

Compared to the lowest quartile, the highest quartile of pre-diagnostic levels of estradiol, free estradiol, DHEAS, testosterone and free testosterone was significantly associated with a 1.4-2.0-fold increased breast cancer risk (Table 2); the associations were similar whether the hormones were measured within 10 years or 10-20 years before diagnosis, (range, $p_{\text{heterogeneity}}$=0.2 (free testosterone) to 0.7 (estradiol)). For example, for estradiol, the top versus bottom quartile contrast was 2.0 (95%CI: 1.4-2.7; p_{trend}<0.001) for <10 years, 2.0 (95%CI: 1.3-3.1; p_{trend}=0.002) for >10-20 years before diagnosis. Although based on fewer cases, associations were similar for 16-20 years before diagnosis; same category contrast RR=2.2 (95%CI:1.1-4.7, p_{trend}=0.03) for estradiol, RR=1.9 (95%CI:0.9-3.9, p_{trend}=0.38) for testosterone, RR=1.8 (95%CI:0.8-4.1, p_{trend}=0.13) for DHEAS, and RR=0.51(95%CI: 0.26-0.98, p_{trend}=0.06) for SHBG (Supplementary Table 1).

In the analysis combining all follow-up years, we observed significant trends for increasing risk (p_{trend}<0.001) associated with estradiol (RR, top vs. bottom quartile=2.1, 95%CI: 1.6-2.7) and free estradiol (comparable RR=1.9, 95%CI: 1.4-2.4) (Table 3). Similar, though weaker associations were observed for testosterone (RR=1.5, 95%CI: 1.2-1.9), free testosterone (RR=1.9, 95%CI: 1.5-2.5), and DHEAS (RR=1.7, 95%CI: 1.3-2.3). There was an inverse association for SHBG (RR=0.68, 95%CI: 0.52-0.88). The magnitude of association was similar for in situ and invasive breast cancer ($p_{\text{heterogeneity}}$>0.15).

Stronger associations were observed for hormone receptor positive tumors. For example, higher estradiol levels were associated with an increased risk of ER+/PR+ tumors (highest vs. lowest quartile RR=2.8, 95%CI: 2.0-4.0, p_{trend}<0.001), but not with ER-/PR- tumors (comparable RR=1.1, 95%CI: 0.6-2.1, p_{trend}=0.98), while associations with ER+/PR- tumors were intermediate (comparable RR=1.3, 95%CI: 0.7-2.5, p_{trend} =0.34). Similar results were observed for free estradiol. For testosterone and free testosterone, we observed a significant

2-fold increased risk for both ER+/PR+ and ER+/PR- tumors when comparing the highest to lowest quartiles, but no suggestion of an association for ER-/PR- tumors.

The associations with each hormone generally were similar ($p_{heterogeneity}$>0.15) when we considered other tumor characteristics (ductal vs. lobular, tumor size <2 cm vs. ≥2 cm, moderately to well vs. poorly differentiated, lymph node positive vs. negative; data not shown). We further evaluated the associations among the subset of cases that recurred or resulted in death (n=85); all RRs were similar to or stronger than those observed for overall breast cancer (Table 3). For estradiol, the RR of recurrent or fatal breast cancer among women in the top (vs. bottom) quartile of levels was 2.6 (95%CI: 1.3-5.1). Among the small group of ER+ recurrent or fatal tumors (n=53), the association with estradiol appeared very strong (comparable RR=6.0, 95%CI: 2.2-18, p_{trend} <0.001).

Finally, we considered including two hormones (or one hormone and BMI) in the same model. The RRs for each hormone (or current BMI) attenuated after mutual adjustment, although the associations largely remained statistically significant (Table 4). For example the RRs comparing extreme quartiles for estradiol without and with adjusting for testosterone were 2.1 and 1.9, respectively; however the comparable RRs for testosterone without and with adjusting for estradiol were 1.5 and 1.2 (not significant), respectively. Results for ER+/PR+ cases were similar.

Discussion

Previous cohort studies, including our earlier report in the NHS, have demonstrated increased breast cancer risk with elevated postmenopausal blood concentrations of estrogens and androgens. Our current study further supports that a single blood sex hormone measurement can predict breast cancer risk for up to16- 20 years prior to breast cancer diagnosis, with statistically significant positive associations observed for estradiol, free estradiol, testosterone, free testosterone, and DHEAS, and a significant inverse association with SHBG. Hormone levels were more strongly associated with aggressive disease, defined here as recurrent or fatal breast cancer, and with hormone receptor positive breast tumors.

Prior prospective studies assessing hormones and breast cancer risk by time since blood collection focused on whether preclinical disease influenced the observed associations, observing that positive associations with higher levels of hormone measurements persisted after excluding breast cancer cases occurring within the first 2 years of blood collection [3, 25, 29, 40]. However, few studies have had follow-up periods greater than 10 years, with only two previous studies evaluating whether one measurement of sex hormones predicted long-term risk. The first study reported stronger positive associations with urinary estradiol measured <10 years (top vs. bottom quartile RR=1.9) versus >10 years (comparable RR=1.2) before diagnosis of breast cancer. A similar pattern was observed for testosterone. In the NYU Women's Health Study, associations over 13 years were similar to associations excluding the first 5 years of follow-up. Our findings were consistent with the second study and suggest that the sex hormones measured 10-20 years before breast cancer diagnosis are significantly associated with postmenopausal breast cancer risk. Moreover, we observed fairly high temporal reliability of these hormones (i.e., 10-year ICCs 0.5-0.7). Collectively, if the inclusion of plasma hormone levels improves the discriminatory ability of breast cancer risk prediction models [19, 37], as indicated in our initial analyses [38], the current data suggest that hormone levels would not need to be measured more than once every 10, or possibly 20, years.

Although the positive association between endogenous sex hormones and breast cancer risk is established, it remains unclear to what extent these associations differ by tumor subtypes.

We observed that women with higher levels of estradiol had a significantly increased risk of hormone receptor positive tumors only, consistent with previous studies [3, 11, 16, 25, 27, 40, 46]. For example, a 2 to 2.6-fold higher risk with estradiol was observed for ER+ breast tumors in the Women's Health Initiative (WHI) [16] and the European Prospective Investigation into Cancer and Nutrition cohort [25]. Interestingly, similar to three prior studies that assessed the associations by ER and PR status [3, 25, 40], we observed a weaker association with ER+/PR- tumors, compared to ER+/PR+ tumors. PR expression may serve as a marker of ER pathway activation [23, 34]. Hence ER+/PR- tumors may have less estrogen pathway activation, and less responsiveness to sex hormones.

The estrogen-breast cancer association has strong biologic plausibility [44]. Estrogens play a key role in the development of normal breast tissue and breast cancer progression [6, 20, 44], through inhibition of apoptosis and stimulation of cell proliferation via ER activation [13]. In addition, hormone-related breast cancer risk factors (e.g., postmenopausal BMI and postmenopausal hormone use) tend to have more pronounced associations with hormone receptor positive tumors [2]. Moreover, drugs that block the binding of estrogen to ER (e.g., tamoxifen [17]) or prevent aromatization of androgens to estrogens (e.g., exemestane [28]) improve survival in women with hormone receptor positive tumors and decrease risk of ER+ tumors [4, 12, 17].

In contrast, the associations with hormone receptor negative tumors have been inconsistent [16, 25]. Our null results for estradiol and ER- tumors were consistent with those observed in the WHI [16]. However, in the largest analysis to date, a non-significant positive association for ER-tumors was reported for estradiol (top vs. bottom tertile RR=1.65; p_{trend}=0.09) [25]. Further evaluation by pooling existing studies is needed.

The mechanisms by which androgens may influence breast cancer remain unclear [7, 14, 31]. Androgens may influence risk indirectly via aromatization to estradiol [7, 14, 31]. Our results for testosterone are consistent with previous studies that have reported positive associations [29], especially for hormone receptor positive subtypes. For hormone receptor negative breast cancer, the WHI [16] noted that higher serum testosterone was significantly associated with a 50% lower risk of ER- breast cancer. Our current study observed a similar, although not statistically significant, pattern. Three other studies [3, 25, 40] observed null or non-significant positive associations. In addition, we observed that adjustment for estradiol significantly attenuated the risk estimates for testosterone. These findings, consistent with most [3, 16, 26, 29, 45] but not all [40] studies, favor the argument that the positive association between higher testosterone level and risk of postmenopausal breast cancer is at least partly attributed to its conversion to estrogen by aromatase. For DHEAS, we observed a significant positive association that is consistent with the re-analysis of nine cohort studies and two other studies published subsequently [3, 45]. Additional studies are needed to evaluate the effect of androgens on breast cancer independent of the known cancer-promoting estrogen effect.

Besides the potential role as an estrogen precursor, testosterone and DHEAS may affect breast cancer risk through the androgen receptor (AR) although, to our knowledge, no previous study has examined hormone levels and breast cancer risk by tumor AR status. Compared to ER- tumors, ER+ tumors are more likely to be AR+ [8, 24, 35], and these proportions are similar across PR status [8, 24, 35]. Therefore ER positivity might crudely reflect an association with AR+ tumors. This notion is supported by the observation of significant associations with both ER+/PR- and ER+/PR+ for testosterone that for estradiol were seen only for ER+/PR+. Although data from other cohorts is limited, generally similar findings were seen in two studies [25, 40], but not a third [3]. Future studies directly examining androgen levels and risk of breast cancer by AR status will be important.

Since estrogens and androgens bind to SHBG, increased levels of SHBG may decrease risk of breast cancer. Consistent with prior studies [3, 26, 29, 45], we observed an inverse association. We also observed that the significant inverse associations were stronger for ER +/PR+ tumors. Although no consistent pattern was observed for SHBG and tumor subtypes among four prior studies [3, 25, 40, 45], three had less than 50 ER- tumors.

Our study has several strengths. Our 20-year follow-up and large number of cases allowed a detailed analysis of the temporal relationship between a single blood hormone and subsequent breast cancer risk. We also collected detailed tumor-specific data, and used high quality assays, conducted with excellent precision. The limitations of our study merit consideration. Our results may not be generalized to other ethnic groups and postmenopausal women who use PMH. Although our study was large overall, case numbers in several groups (e.g., ER-/PR-) were relatively small, and we had insufficient power to assess other relevant breast cancer subtypes [18], such as triple negative, basal-like, or HER2+.

In summary, our study demonstrated that plasma levels of estradiol, testosterone, DHEAS, and SHBG measured up to 16-20 years before breast cancer diagnosis were significantly associated with risk of postmenopausal breast cancer among women not using PMH. Importantly, these associations were stronger for ER+/PR+ tumors. Additional large studies on postmenopausal women using PMH, premenopausal women, on less common but more aggressive molecular subtypes (e.g., triple negative, HER2+, basal-like) and by AR status will be informative.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institute of Health grants CA49449 and CA87969. We appreciate the participants and staff of the Nurses' Health Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY.

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Table 1

Characteristics of cases and matched controls at each blood collection in the Nurses' Health Study

	1989-1990 blood draw			2000-2002 blood draw		
	Cases	**Controls**	***p*-value***	**Cases**	**Controls**	***p*-value***
No. of cases/controls	641	1287		155	296	
Age at blood draw, yr, Mean (SD)	61.2(4.8)	61.2(4.8)	0.76	68.4(6.7)	68.6(6.6)	0.81**
BMI at blood draw, kg/m^2, Mean (SD)	26.6(4.9)	26.1(4.7)	0.03	27.8(5.5)†	27.0(5.9)	0.13
Age of menarche, yr, Mean (SD)	12.6(1.4)	12.6(1.5)	0.71			
Age of first birth, yr, Mean (SD)	32.2(20.7)	30.7(18.8)	0.13			
Age of menopause, yr, Mean (SD)	50.0(3.9)	49.7(4.2)	0.07			
BMI at age 18, kg/m^2, Mean (SD)	21.5(3.0)	21.7(3.1)	0.20			
Parous (%)	88	90	0.17			
Breast benign diseases (%)	38	32	0.01	59	49	0.05
Family history of breast cancer (%)	19	14	0.008	29	22	0.10
Alcohol consumption, g/d, Mean (SD)	5.0(9.1)	5.0(9.3)	0.95	5.5(10.1)	5.2(8.8)	0.74
Estradiol (pg/mL), Mean (SD)	8.8(7.8)	7.3(5.5)	<0.001	8.6(6.2)	6.9(5.2)	0.005
Free estradiol (pg/mL), Mean (SD)	0.13(0.12)	0.10(0.08)	<0.001	0.13(0.10)	0.10(0.09)	0.01
Testosterone (ng/dL), Mean (SD)	24.2(14.1)	22.0(12.1)	0.001	26.9(17.3)	25.6(16.5)	0.47
Free testosterone (ng/dL), Mean (SD)	0.24(0.21)	0.20(0.15)	<0.001	0.28(0.26)	0.24(0.18)	0.15
DHEAS (μg/dL), Mean (SD)‡	82.4(55.9)	71.6(48.5)	<0.001	71.8(58.7)	67.5(61.9)	0.49
SHBG (nmol/L), Mean (SD)	60.3(35.1)	63.4(31.3)	0.06	55.0(25.2)	60.6(30.7)	0.04

* *p*-values were calculated using mixed-effects model comparing cases with controls adjusting for matching factors.

** Matching factor

† Same values for both blood collections; a total of 267 women (89 cases and 178 controls) had two bloods.

‡ There were 615 cases/949 controls for the 1989-1990 blood draw; and 129 case/151 controls for the 2000-2002 blood draw.

Table 2

Multivariable relative risks* of overall breast cancer in relation to pre-diagnostic hormone levels in the Nurses' Health Study, by years from blood collection to diagnosis

Hormones	Hormone levels before diagnosis (quartiles)				*p*-trend	*p-int. by time*
	Q1	Q2	Q3	Q4		
Estradiol (pg/mL)	≤4	>4-6	>6-9	>9		
No. cases/controls	92/256	138/301	122/239	186/272		
≤10 yrs†	1.0 (ref.)	1.3 (0.9-1.8)	1.5 (1.0-2.0)	2.0 (1.4-2.7)	<0.001	0.7
No. cases/controls	73/204	61/124	71/107	53/80		
>10-20 yrs†	1.0 (ref.)	1.4 (0.9-2.2)	1.9 (1.2-2.8)	2.0 (1.3-3.1)	0.002	
Free estradiol (pg/mL)	≤0.05	>0.05-0.08	>0.08-0.13	>0.13		
No. cases/controls	78/224	119/264	136/276	205/304		
≤10 yrs†	1.0 (ref.)	1.3 (0.9-1.8)	1.5(1.0-2.1)	2.0 (1.5-2.8)	<0.001	0.7
No. cases/controls	66/184	59/125	75/111	58/95		
>10-20 yrs†	1.0 (ref.)	1.4 (0.9-2.1)	1.9 (1.2-2.9)	1.8 (1.2-2.8)	0.01	
Testosterone (ng/dL)	≤14	>14-19	>19-26.9	>26.9		
No. cases/controls	116/260	97/260	153/276	172/272		
≤10 yrs†	1.0 (ref.)	0.8 (0.6-1.1)	1.3 (0.9-1.7)	1.4 (1.1-1.9)	0.001	0.3
No. cases/controls	50/150	58/109	73/101	77/155		
>10-20 yrs†	1.0 (ref.)	1.6 (1.0-2.5)	2.2 (1.4-3.5)	1.6 (1.0-2.4)	0.11	
Free testosterone (ng/dL)	≤0.11	>0.11-0.17	>0.17-0.24	>0.24		
No. cases/controls	84/252	134/260	124/257	196/299		
≤10 yrs†	1.0 (ref.)	1.6 (1.2-2.2)	1.5 (1.1-2.1)	2.0 (1.5-2.8)	<0.001	0.2
No. cases/controls	48/130	55/121	74/127	81/137		
>10-20 yrs†	1.0 (ref.)	1.3 (0.8-2.0)	1.7 (1.1-2.6)	1.8 (1.2-2.8)	0.01	
DHEAS (μg/dL)	≤37.2	>37.2-59.6	>59.6-95.9	>95.9		
No. cases/controls	106/234	137/201	115/200	149/194		
≤10 yrs†	1.0 (ref.)	1.7 (1.2-2.3)	1.4 (1.0-2.0)	1.9 (1.3-2.6)	0.003	0.3

Hormones	Hormone levels before diagnosis (quartiles)				*p*-trend	*p-int. by time*
	Q1	**Q2**	**Q3**	**Q4**		
No. cases/controls	45/58	45/64	66/70	81/79		
>10-20 yrs†	1.0 (ref.)	0.9 (0.5-1.5)	1.2 (0.7-2.0)	1.2 (0.7-2.1)	0.22	
SHBG (nmol/L)	≤40.7	>40.7-58.0	>58.0-81.3	>81.3		
No. cases/controls	177/269	130/292	121/250	110/257		
≤10 yrs†	1.0 (ref.)	0.68 (0.51-0.91)	0.72 (0.54-0.97)	0.63 (0.47-0.85)	0.006	0.9
No. cases/controls	81/136	63/112	58/139	56/128		
>10-20 yrs†	1.0 (ref.)	0.92 (0.60-1.42)	0.68 (0.45-1.04)	0.68 (0.44-1.04)	0.04	

>10-20 yrs = blood sample drawn >10-20 years prior to diagnosis in cases and matched time period for controls (258ca/515co); 237ca/271co for DHEAS.

*Multivariable models adjusted for: fasting status (<8 hours, ≥ 8 hours), time of day (24 hour clock: <8, 8-12, 13-24), age at blood draw (continuous), season of blood draw (May to October, other months), BMI at age 18 (<21,21 -<23, ≥23 kg/m^2), history of benign breast disease (no, yes), family history of breast cancer (no, yes), age at menopause (continuous), age at menarche (<12,12,13, or ≥14 y), physical activity (<3, 3-27,>27 MET-hrs/wk), and age at first birth and parity (nulliparous; 1-4children, first birth<25 y; 1-4 children, first birth 25-29 y; 1-4 children, first birth ≥30 y; ≥ 5 children, first birth<25 y; or ≥5children, first birth ≥25 y).

†≤ 10 yrs = blood sample drawn ≤ 10 years prior to diagnosis in cases and matched time period for controls (538ca/1068co); 507ca/829co for DHEAS.

Table 3

Multivariable relative risks* of postmenopausal breast cancer subtypes in relation to pre-diagnostic hormone levels in the Nurses' Health Study

Hormones		Quartile categories of hormone levels*				*p*-trend	*p-heterogeneity* ‡
		Q1	Q2	Q3	Q4		
Estradiol	**Overall**	1.0(ref.)	1.4 (1.1-1.8)	1.5 (1.1-2.0)	2.1 (1.6-2.7)	<0.001	
	Insitu	1.0(ref.)	1.7 (0.9-3.1)	1.1 (0.6-2.2)	2.0 (1.1-3.7)	0.08	0.65
	Invasive	1.0(ref.)	1.3 (1.0-1.8)	1.6 (1.2-2.1)	2.1 (1.6-2.7)	<0.001	
	ER+/PR+	1.0(ref.)	1.5 (1.0-2.2)	1.9 (1.3-2.7)	2.8 (2.0-4.0)	<0.001	0.007
	ER+/PR-	1.0(ref.)	1.1 (0.6-2.0)	1.9 (1.0-3.4)	1.3 (0.7-2.5)	0.34	
	ER-/PR-	1.0(ref.)	1.2 (0.6-2.1)	0.9 (0.4-1.7)	1.1 (0.6-2.1)	0.98	
	Fatal/Recurred	1.0(ref.)	1.7 (0.8-3.4)	2.3 (1.2-4.7)	2.6 (1.3-5.1)	0.01	
Free estradiol	**Overall**	1.0(ref.)	1.2 (0.9-1.6)	1.4 (1.1-1.9)	1.9 (1.4-2.4)	<0.001	
	Insitu	1.0(ref.)	0.9 (0.5-1.8)	0.9 (0.5-1.7)	1.2 (0.7-2.2)	0.44	0.22
	Invasive	1.0(ref.)	1.3 (0.9-1.7)	1.6 (1.2-2.1)	2.0 (1.9-2.6)	<0.001	
	ER+/PR+	1.0(ref.)	1.3 (0.9-1.9)	2.1 (1.4-3.0)	2.8 (1.9-4.0)	<0.001	<0.001
	ER+/PR-	1.0(ref.)	1.0 (0.5-1.8)	1.1 (0.6-2.0)	1.0 (0.5-1.8)	0.95	
	ER-/PR-	1.0(ref.)	1.4 (0.8-2.7)	1.0 (0.5-2.0)	1.0 (0.5-2.0)	0.56	
	Fatal/Recurred	1.0(ref.)	2.4 (1.1-5.2)	2.4 (1.1-5.4)	3.1 (1.4-6.7)	0.01	
Testosterone	**Overall**	1.0(ref.)	1.0 (0.7-1.3)	1.5 (1.2-2.0)	1.5 (1.2-1.9)	<0.001	
	Insitu	1.0(ref.)	1.3 (0.7-2.6)	1.9 (1.0-3.6)	2.0 (1.0-3.7)	0.03	0.78
	Invasive	1.0(ref.)	0.9 (0.7-1.3)	1.4 (1.1-1.9)	1.5 (1.1-1.9)	<0.001	
	ER+/PR+	1.0(ref.)	1.1 (0.7-1.5)	1.6 (1.1-2.2)	1.8 (1.3-2.5)	<0.001	0.02
	ER+/PR-	1.0(ref.)	1.4 (0.7-2.8)	2.3 (1.2-4.5)	2.0 (1.0-3.9)	0.05	
	ER-/PR-	1.0(ref.)	0.5 (0.3-1.1)	0.8 (0.5-1.5)	0.6 (0.3-1.2)	0.35	
	Fatal/Recurred	1.0(ref.)	1.2 (0.6-2.5)	2.0 (1.1-3.8)	1.3 (0.7-2.6)	0.43	
Free testosterone	**Overall**	1.0(ref.)	1.4 (1.0-1.8)	1.5 (1.1-2.0)	1.9 (1.5-2.5)	<0.001	
	Insitu	1.0(ref.)	2.0 (1.1-3.9)	2.0 (1.0-3.9)	1.6 (0.8-3.2)	0.52	0.17
	Invasive	1.0(ref.)	1.3 (1.0-1.7)	1.5 (1.1-2.0)	2.0 (1.5-2.6)	<0.001	
	ER+/PR+	1.0(ref.)	1.3 (0.9-2.0)	1.9 (1.3-2.7)	2.6 (1.8-3.8)	<0.001	0.008

Hormones		Quartile categories of hormone levels*				*p*-trend	*p-heterogeneity* ‡
		Q1	Q2	Q3	Q4		
	ER+/PR-	1.0(ref.)	1.6 (0.8-3.1)	1.7 (0.8-3.3)	1.9 (0.9-3.7)	0.07	
	ER-/PR-	1.0(ref.)	0.9 (0.4-1.7)	0.7 (0.4-1.4)	0.9 (0.5-1.7)	0.55	
	Fatal/Recurred	1.0(ref.)	1.0 (0.5-2.1)	1.3 (0.7-2.6)	1.8 (1.0-3.5)	0.03	
DHEAS	**Overall**	1.0(ref.)	1.4 (1.1-1.9)	1.4 (1.1-1.9)	1.7 (1.3-2.3)	0.001	
	Insitu	1.0(ref.)	1.6 (0.8-3.3)	1.9 (0.9-3.8)	1.3 (0.6-2.8)	0.69	0.36
	Invasive	1.0(ref.)	1.4 (1.0-1.9)	1.4 (1.0-1.9)	1.7 (1.3-2.4)	0.001	
	ER+/PR+	1.0(ref.)	1.6 (1.1-2.3)	1.8 (1.2-2.6)	2.1 (1.4-3.0)	<0.001	0.25
	ER+/PR-	1.0(ref.)	1.2 (0.6-2.4)	1.0 (0.5-2.0)	1.7 (0.9-3.3)	0.10	
	ER-/PR-	1.0(ref.)	0.9 (0.5-1.8)	0.6 (0.2-1.2)	1.1 (0.6-2.0)	0.94	
	Fatal/Recurred	1.0(ref.)	4.8 (2.1-11.2)	2.9 (1.2-7.0)	3.9 (1.6-9.3)	0.06	
SHBG	**Overall**	1.0(ref.)	0.80 (0.62-1.02)	0.77 (0.59-0.99)	0.68 (0.52-0.88)	0.004	
	Insitu	1.0(ref.)	0.99 (0.53-1.87)	1.68 (0.94-2.99)	1.02 (0.54-1.94)	0.65	0.50
	Invasive	1.0(ref.)	0.77 (0.60-1.01)	0.66 (0.50-0.86)	0.65 (0.50-0.86)	0.002	
	ER+/PR+	1.0(ref.)	0.82 (0.61-1.11)	0.58 (0.41-0.80)	0.43 (0.30-0.62)	<0.001	0.003
	ER+/PR-	1.0(ref.)	1.09 (0.59-2.02)	0.89 (0.46-1.71)	1.14 (0.62-2.12)	0.77	
	ER-/PR-	1.0(ref.)	0.62 (0.32-1.21)	0.69 (0.36-1.33)	1.08 (0.58-1.98)	0.60	
	Fatal/Recurred	1.0(ref.)	0.71 (0.38-1.30)	0.70 (0.38-1.31)	0.73 (0.40-1.34)	0.35	

*See Table 2 for covariates included in models and for hormone quartile category definitions. A total of 1405 controls, 707 overall breast cancer cases, 95 in situ cases, 598 invasive cases, 347 ER+/PR+ cases, 86 ER+/PR- cases, 80 ER-/PR- cases, 85 fatal/recurred cases were included in the analysis. For DHEAS, overall, we have 672 cases and 1016 controls.

‡*p*heterogeneity was calculated by comparing the trend across case groups.

Table 4

Multivariable relative risks of breast cancer comparing top vs. bottom quartiles of hormone levels after mutual adjustment in the Nurses' Health Study

Hormones	Models	Overall breast cancer	*p*-trend	ER+/PR+ breast cancer	*p*-trend
Estradiol†	MV*	2.1 (1.6-2.7)	<0.001	2.8 (2.0-4.0)	<0.001
	MV+ testosterone	1.9 (1.4-2.5)	<0.001	2.5 (1.7-3.7)	<0.001
	MV+ free testosterone	1.7 (1.3-2.4)	0.001	2.0 (1.3- 3.1)	0.001
	MV+ SHBG	2.0 (1.5-2.6)	<0.001	2.3 (1.6- 3.4)	<0.001
	MV+ current BMI	2.0 (1.5-2.7)≠	<0.001	2.5 (1.7- 3.8)	<0.001
Free estradiol†	MV*	1.9 (1.4-2.4)	<0.001	2.8 (1.9-4.0)	<0.001
	MV+ testosterone	1.7 (1.3-2.2)	<0.001	2.5 (1.7-3.6)	<0.001
	MV+ free testosterone	1.5 (1.1-2.1)	0.01	2.0 (1.3- 3.1)	0.001
	MV+ SHBG	1.9 (1.4-2.7)	<0.001	2.2 (1.4- 3.4)	<0.001
	MV+ current BMI	1.8 (1.3-2.5)	<0.001	2.6 (1.7- 3.9)	<0.001
Testosterone†	MV*	1.5 (1.2-1.9)	<0.001	1.8 (1.3-2.5)	<0.001
	MV+ estradiol	1.2 (0.9-1.6)	0.07	1.3 (0.9-1.8)	0.11
	MV+ free estradiol	1.3 (1.0-1.7)	0.01	1.4 (1.0-2.0)	0.03
	MV+ SHBG	1.6(1.2-2.1)	<0.001	2.0 (1.4-2.8)	<0.001
	MV+ current BMI	1.5 (1.1-1.9)	<0.001	1.7 (1.2-2.4)	<0.001
Free testosterone†	MV*	1.9 (1.5-2.5)	<0.001	2.6 (1.8-3.7)	<0.001
	MV+ estradiol	1.5 (1.1-2.0)	0.02	1.8 (1.2- 2.8)	0.003
	MV+ free estradiol	1.5 (1.1-2.1)	0.03	1.7 (1.1- 2.6)	0.01
	MV+ SHBG	1.9 (1.4-2.5)	<0.001	2.0 (1.3-3.0)	<0.001
	MV+ current BMI	1.8 (1.4-2.4)	<0.001	2.3 (1.6-3.4)	<0.001
DHEAS	MV*	1.7 (1.3-2.3)	0.001	2.1 (1.4-3.0)	<0.001
	MV+ estradiol	1.5 (1.1-2.0)	0.04	1.7 (1.1-2.5)	0.02
	MV+ free estradiol	1.5 (1.1-2.0)	0.03	1.6 (1.1-2.4)	0.03
	MV+ testosterone	1.5 (1.1-2.1)	0.03	1.8 (1.2-2.7)	0.01
	MV+ free testosterone	1.4 (1.0-1.9)	0.07	1.6 (1.1-2.4)	0.058

Hormones	Models	Overall breast cancer	*p*-trend	ER+/PR+ breast cancer	*p*-trend
	MV+ current BMI	1.7 (1.3-2.3)	0.001	2.1 (1.4-3.0)	0.001
SHBG	MV*	0.68 (0.52-0.88)	0.004	0.43 (0.30-0.62)	<0.001
	MV+ estradiol	0.81 (0.62-1.07)	0.18	0.55 (0.38-0.80)	0.001
	MV+ free estradiol	0.95 (0.69-1.30)	0.86	0.69 (0.45-1.06)	0.06
	MV+ testosterone	0.63 (0.48-0.82)	0.001	0.40 (0.28-0.57)	<0.001
	MV+ free testosterone	0.91 (0.67-1.22)	0.61	0.61 (0.41-0.92)	0.01
	MV+ current BMI	0.74 (0.56-0.98)	0.046	0.49 (0.34-0.73)	<0.001

*MV: multivariable adjustment, we adjusted the same factors as shown in Table 2 footnotes.

†We have not adjusted for estradiol and free estradiol mutually because of the high correlations (r=0.93) between these 2 hormones. Similarly, we have not adjusted for testosterone and free testosterone mutually (r=0.73).

≠For overall breast cancer, RRs (95%CIs) for current BMI (≥30 vs. <25 kg/m^2) changed from 1.36 (1.06, 1.75) to 1.00 (0.91 -1.40) after adjustment for estradiol, 1.01 (0.76-1.36) after adjustment for free estradiol, 1.34 (1.04-1.73) after adjust for testosterone, 1.17 (0.90-1.52) after adjust for free testosterone, 1.23 (0.94-1.61) after adjust for SHBG, 1.29 (0.98-1.69) after adjust for DHEAS.