Reproductive Steroid Hormones and Recurrence-Free Survival in Women with a History of Breast Cancer

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Abstract

Epidemiologic studies fairly consistently show in postmenopausal women that reproductive steroid hormones contribute to primary breast cancer risk, and this association is strongly supported by experimental studies using laboratory animals and model systems. Evidence linking sex hormone concentrations with risk for recurrence in women diagnosed with breast cancer is limited; however, beneficial effects of antiestrogenic therapy on recurrence-free survival suggest that these hormones affect progression and risk for recurrence. This study examined whether baseline serum concentrations of estradiol, testosterone, and sex hormone binding globulin were associated with recurrence-free survival in a nested case-control cohort of women from a randomized diet trial (Women's Healthy Eating and Living Study) who were followed for >7 years after diagnosis. In 153 case-control pairs of perimenopausal and postmenopausal women in this analysis, total estradiol [hazard ratio (HR), 1.41 per unit increase in log concentration; 95% confidence interval (95% CI), 1.01-1.97], bioavailable estradiol (HR, 1.26; 95% CI, 1.03-1.53), and free estradiol (HR, 1.31; 95% CI, 1.03-1.65) concentrations were significantly associated with risk for recurrence. Recurred women had an average total estradiol concentration that was double that of non-recurred women (22.7 versus 10.8 pg/mL; $P = 0.05$). Testosterone and sex hormone binding globulin concentrations did not differ between cases and controls and were not associated with risk for recurrence. Although genetic and metabolic factors likely modulate the relationship between circulating sex hormones and risk, results from this study provide evidence that higher serum estrogen concentration contributes to risk for recurrence in women diagnosed with early stage breast cancer. (Cancer Epidemiol Biomarkers Prev 2008;17(3):614–20)

Introduction

Estrogenic stimulation is believed to play a causal role in the pathogenesis of breast cancer (1, 2), and laboratory animal experiments have shown that estrogens promote breast tumorigenesis (3). Serum concentrations of endogenous sex hormones are strongly associated with risk for primary breast cancer in postmenopausal women (4, 5). Estradiol is the major determinant of the mitotic rate of breast epithelial cells (6), which may explain the association between increased estrogen exposure and risk for breast cancer, although another possible mechanism may involve the damaging effect of oxidative metabolites of estrogen on DNA (1). Data on the relationship between circulating estrogen concentration and breast cancer risk in premenopausal women in epidemiologic studies are difficult to interpret due in part to fluctuations in these concentrations over the menstrual cycle.

Few studies have investigated the relationship between circulating estrogen concentrations and recurrence-free survival among women who have been diagnosed with breast cancer. An important consideration in this group is the potential effect of cancer treatment on serum estrogens; for example, many women who were premenopausal at their diagnosis of breast cancer experience ovulatory failure in association with initial treatments. Nonetheless, minimizing estrogen stimulation following the diagnosis of breast cancer is a standard management strategy. In fact, antiestrogen therapy has emerged as one of the most effective treatments of endocrine-responsive breast cancers, which account for approximately two-thirds of cases as shown in randomized clinical trials (7, 8). Antiestrogenic medications have variable effects on reproductive steroid hormones and related factors in women who have been diagnosed with breast cancer. Tamoxifen promotes

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increased concentrations of sex hormone binding globulin (SHBG) and estradiol (9, 10), whereas aromatase inhibitors reduce serum estrogens and have minimal effect on SHBG concentration in nonobese breast cancer survivors (11).

Serum concentrations of androgens, particularly androstenedione and testosterone, are also directly related to breast cancer risk in postmenopausal women (4). These hormones can be readily converted to estrone and estradiol, respectively. Also, they are typically present in concentrations higher than those of estrogens in postmenopausal women. Increased urinary testosterone concentration has been associated with poor outcome in women diagnosed with breast cancer (12), and higher serum testosterone concentration was a strong negative prognostic factor for new breast cancer events (contralateral breast cancer, distant metastasis, and local relapse) during 5.5 years of follow-up in a sample of 110 postmenopausal women who had been diagnosed and treated for breast cancer (13).

This analysis examines whether circulating concentrations of selected reproductive steroid hormones (total, bioavailable, and free estradiol and total, bioavailable, and free testosterone) and SHBG were associated with risk for recurrence in participants in the Women's Healthy Eating and Living (WHEL) Study, a dietary intervention trial. Using a nested case-control design, we examined whether higher baseline serum concentrations of estradiol and testosterone would be associated with increased likelihood of recurrence in this study sample.

Materials and Methods

Study Participants. This paired analysis employs a nested case-control design within the WHEL Study, comparing cases (who recurred or experienced a new breast cancer) with matched controls who were recurrence free as described below. The WHEL Study is a multisite randomized trial that tested the effect of an intensive dietary intervention on disease-free survival in a cohort of women diagnosed previously with early-stage breast cancer who were recruited between 1995 and 2000. Details of the study design, protocol, and overall effect of the dietary intervention on outcome during the 7.3-year follow-up period have been reported previously (14, 15). Eligibility criteria included evidence from the medical record of a diagnosis within the past 4 years of primary operable invasive breast carcinoma categorized using the American Joint Committee on Cancer criteria (16) as stage I tumor ($\geq$1 cm), stage II, stage IIIA, or stage IIIC; ages 18 to 70 years at diagnosis; treated with axillary dissection and total mastectomy or lumpectomy followed by primary breast radiation; not scheduled for or currently undergoing chemotherapy; no evidence of recurrent disease or new breast cancer since completion of initial local treatment; and no other invasive cancer in the past 10 years. Participants were recruited from seven clinical sites. Data on the original breast cancer diagnosis (date of diagnosis, tumor stage, grade, and estrogen receptor status) were reviewed and confirmed via medical record. Further, medical records for each reported breast cancer recurrence or new primary breast cancer diagnosed after study enrollment were reviewed and confirmed by two oncologists as reported previously (14, 15).

Relevant to this analysis, the protocol involved a clinic visit at enrollment and specified intervals, at which a fasting blood sample was collected; height, weight, and waist and hip circumferences were measured using standard procedures; and body mass index [weight (kg) / height (m^2)] was computed. Additional data collected included demographic characteristics, self-reported menopausal status, history of bilateral oophorectomy, and adjuvant antiestrogen use. The institutional review boards of all the participating institutions approved procedures for this study, and written informed consent was obtained from all study participants before their enrollment.

Nested Case-Control Design. Within the WHEL Study cohort, each woman with a new breast cancer event was matched within a nested case-control design with a participant who was disease free. The matching strategy followed the work of Langholz and Thomas (17) and Lubin and Gail (18). Each recurrence (case) in year N was matched with another WHEL Study participant who was observed to be cancer free (control) after N years. Controls could be matched with more than one case, and women who subsequently recurred could be controls for women who recurred earlier. Matching criteria were clinical site, cancer stage, age at cancer diagnosis, date of cancer diagnosis, and date of randomization into the WHEL Study. Most matches (67% for date of diagnosis and 88% for date randomized) were within 12 months of each other, and virtually all matches were within 3 years.

Although the present analysis focuses exclusively on baseline hormone concentrations, any recurred woman for whom either she or her match was missing a 1-year blood sample was excluded from this cohort. Therefore, 345 women (representing 188 recurrences, each paired with a nonrecurred match) constituted the initial cohort.

Dietary, Physical Activity, and Additional Data. Dietary fiber intake was included in the analysis because we have observed previously an inverse association between dietary fiber intake and serum estradiol concentration in a subsample of WHEL Study participants (19), and an inverse association between fiber intake and serum estrogens has been reported in other populations (20). The primary method of dietary assessment consisted of repeated 24-h dietary recalls, described in detail elsewhere (14). Briefly, each study participant provided four 24-h dietary recalls including two weekdays and two weekend days over a 3-week period. Trained dietary assessors, who were blinded to the intervention or comparison group assignment of the participants, collected these data during telephone interviews. Nutrient calculations were done using the Nutrition Data System for Research software, developed by Nutrition Coordinating Center, University of Minnesota (Food and Nutrient Database 31, version 4.03, released November 2000).

Physical activity level has been found to be independently associated with survival after breast cancer diagnosis (21, 22) and was examined as a potential covariate in this analysis. In the WHEL Study, the frequency, duration, and intensity of physical activity were assessed by questionnaire and converted into metabolic equivalents (MET), and the measure was validated in a subsample of the WHEL Study

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participants (23). Total energy expenditure was obtained by weighting time spent per week by METs: mild physical activity was weighted 3 METs, moderate activity was weighted 5 METs, and vigorous activity was weighted 8 METs. Walking METs were assigned by walking speed. Unknown speed or 2 mph walking was weighted 2 METs, 3 mph walking was weighted 3 METs, 4 mph walking was weighted 4 METs, and >5 mph walking was weighted 6 METs (24). METs were not assigned for hours spent sitting or sleeping.

We have observed previously an inverse association between reported hot flashes and risk of breast cancer recurrence in WHEL Study participants assigned to the comparison group and taking tamoxifen (25). The WHEL Study assessed the occurrence and severity of hot flashes with a 34-item self-report symptom inventory that addresses a variety of physical and psychologic symptoms, including vasomotor symptoms, as described in detail elsewhere (14, 25, 26). In this study, we examined hot flashes at baseline as a covariate and also compared serum hormone concentrations in participants reporting hot flashes with those not reporting hot flashes.

Laboratory Analysis. Baseline blood samples were used to measure estradiol, testosterone and SHBG in the Reproductive Endocrine Research Laboratory of Frank Z. Stanczyk at the University of Southern California. Serum concentrations of total estradiol and testosterone were measured by RIA after organic solvent extraction and celite column chromatography; procedural losses were monitored by addition of tritiated standard to each sample before the extraction. These purification steps are critical for the measurement of samples from postmenopausal women because direct assays of estradiol and testosterone lack sufficient sensitivity. The sensitivities for testosterone and estradiol were 20 pg/mL and 4 ng/dL, respectively. The intraassay and interassay coefficients of variation ranged from 6% to 9% and 12% to 14%, respectively, at low, medium, and high levels in quality-control samples, spiked charcoal-stripped serum prepared in the Reproductive Endocrine Research Laboratory. Quality-control sample concentrations averaged 17 pg/mL (low), 71 pg/mL (medium), and 147 pg/mL (high) for estradiol and 10 ng/dL (low), 34 ng/dL (medium), and 95 ng/dL (high) for testosterone. Serum SHBG was measured using the Immulite 2000 analyzer and a two-site chemiluminometric sandwich assay (Siemens Medical Solutions Diagnostics); the sensitivity was 0.2 nmol/L; intraassay and interassay coefficients of variation were 6.5% and 8.7%, respectively. Bioavailable and free testosterone and estradiol were calculated using law of mass action equations (27). Total estradiol (or testosterone) includes SHBG-bound, albumin-bound and free hormone; bioavailable estrogen (or testosterone) includes albumin-bound and free hormone; and free estrogen (or testosterone) is free or unbound hormone. Women with estradiol concentrations

Table 1. Characteristics of cases and controls within a nested case-control cohort of the WHEL Study

	Cases (n = 153)	Controls (n = 153)	P
Chemotherapy during initial treatment (%)	76.5	75.8	0.86
Tumor estrogen receptor status (%)			
Estrogen receptor positive	77.1	78.4	0.79
Estrogen receptor negative	21.6	20.3	
Tamoxifen use at baseline (%)	68.0	73.2	0.32
Age, y	55 (8)	55 (7)	0.56
Ethnicity (%)			
White non-Hispanic	83.7	88.9	0.14
Hispanic	7.8	3.9	
Other	8.5	7.2	
Body mass index, kg/m^2	27.9 (5.7)	27.8 (5.7)	0.83
Physical activity METs, min/wk	703 (768)	742 (791)	0.67
Waist/hip circumference ratio	0.81 (0.07)	0.81 (0.09)	0.57
Prior menopausal hormone therapy (%)	54.9	54.3	0.90
Hot flashes at baseline (%)	67.3	68.6	0.89
History of bilateral oophorectomy (%)	13.1	15.7	0.54
Baseline dietary fiber intake, g/d	20.2 (7.0)	21.4 (8.1)	0.14
Time from diagnosis to randomization, mo	23.1 (12.0)	24.6 (11.3)	0.08
Tumor stage (%)*			
I	17.7	17.7	1.00
IIA	32.7	32.7	
IIB	15.7	15.7	
IIIA	21.6	21.6	
IIIC	12.4	12.4	
Tumor grade (%)			
I	8.5	14.4	0.13
II	43.8	38.6	
III	38.6	38.6	
Unspecified	9.1	8.5	
Menopausal status (%)			
Postmenopausal	93.5	92.8	0.80
Perimenopausal	6.5	7.2	
Time from menopause to study entry [mean (SD)], y	9.9 (9.5)	9.4 (8.5)	0.51

NOTE: Continuous variables were tested with paired t test, and categorical variables are tested with McNemar's paired χ^2 test.
*Tumor stage was an exact matching criterion.

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Table 2. Hormone concentrations in case-control pairs

Hormone	Cases (n = 153)		Controls (n = 153)	Paired t test (P)	HR (95% CI)	P
	Mean (SD)	Median (interquartile range)	Mean (SD) pairwise difference			
Estradiol (pg/mL)	22.7 (56.0)	8.2 (5.8-13.3)	-11.8 (51.2)	0.05	1.41 (1.01-1.97)	0.04
Bioavailable estradiol (pg/mL)	12.5 (29.8)	4.64 (2.8-9.1)	-6.4 (26.8)	0.02	1.26 (1.03-1.53)	0.02
Free estradiol (pg/mL)	0.47 (1.12)	0.17 (0.11-0.34)	-0.24 (1.01)	0.02	1.31 (1.03-1.65)	0.03
Testosterone (ng/dL)	30.6 (18.7)	25.8 (19.5-36.7)	-1.8 (24.4)	0.99	1.15 (0.75-1.74)	0.52
Bioavailable testosterone (ng/dL)	12.4 (8.8)	10.7 (7.5-13.7)	-1.7 (10.6)	0.52	1.21 (0.82-1.80)	0.33
Free testosterone (pg/mL)	4.8 (3.4)	4.2 (2.9-5.3)	-0.7 (4.1)	0.53	1.21 (0.82-1.80)	0.33
SHBG (nmol/L)	75.0 (40.7)	68.0 (46.5-93.5)	+5.6 (58.7)	0.24	0.86 (0.56-1.33)	0.51

NOTE: Values shown are mean (SD) and median (interquartile range) for recurred women (cases), median (SD) difference in controls, significance level for paired t tests, HR (95% CI), and significance level for a Cox model of time to event adjusted for tumor grade (153 pairs) in the nested case-control cohort of the WHEL Study.
Paired t tests and Cox models were conducted using log-transformed pairwise data. HRs reflect risk per unit increase in log of hormone concentration.

below the sensitivity of the assay (n = 29) were assigned the value of 3.0 pg/mL for total estradiol and 0.1 pg/mL for bioavailable estradiol.

Statistical Analysis. Premenopausal women (n = 30 individuals in 26 pairs) and those whose estrogen levels exceeded 5 SDs from the mean (n = 9 individuals in 9 pairs), as well as matched pairs for these outliers, were excluded from the present analysis, leaving 153 case-control pairs. Because 13 participants were used as both case and control and 12 of the control women were matched to multiple cases, the paired cohort included 281 unique individuals.

Characteristics of cases were compared with those of controls using paired t tests for continuous variables and McNemar's paired χ^2 tests for categorical variables. Hormone concentrations were log transformed to improve normality. We present medians (interquartile range) as well as means and SDs for cases and mean pairwise differences for controls.

The analysis of time to event (time from study entry to recurrence or censor point) used Cox proportional hazards regression models stratified by case-control pair number to examine the relationship between estrogen, testosterone, and SHBG concentrations on recurrence-free survival. Models were controlled for tumor grade, which has consistently shown a clear association with recurrence. Stage was not included in survival models because all pairs were matched exactly on stage. Any other variables that differed ($P < 0.05$ initially and $P < 0.15$ for subsequent sensitivity analysis) between cases and controls in the McNemar's χ^2 paired tests or paired t tests were considered for inclusion in Cox models. Finally, the association between hot flash status and baseline estrogen concentrations was examined using two-sample t tests on log-transformed data.

Results

Women in the WHEL Study were randomized up to four years after diagnosis (averaging approximately two years) and then followed for an average of 7.3 years. Within this nested case-control design, the mean interval between diagnosis and recurrence was 5.6 years in the 153 cases, with a range of 1.6 to 12.0 years. In a preliminary evaluation of variability in hormone levels, hormone values for 33 self-reported perimenopausal women were compared with those of the postmenopausal women. None of the perimenopausal women had any measured hormone value that was outside the range of values for the postmenopausal women; therefore, the perimenopausal women were included in a preliminary analysis pool. Within the preliminary analysis pool, four postmenopausal and five perimenopausal women were excluded as outliers as described above. The two groups did not have any significant pairwise differences in use of chemotherapy, tumor estrogen receptor status, use of tamoxifen, age, ethnicity, body mass index, physical activity, waist/hip ratio, prior use of menopausal hormone therapy, hot flash status at baseline, previous bilateral oophorectomy, or dietary fiber intake (shown in Table 1). Approximately 93% of each group was postmenopausal, and cases did not differ from controls in mean interval between menopause and study entry (Table 1). Time between diagnosis and study entry averaged 23.1 (12.0) months [mean (SD)] in the recurred group and 24.6 (11.3) months in the matched controls.

As shown in Table 2, serum concentrations of total estradiol [hazard ratio (HR), 1.41; 95% confidence interval (95% CI), 1.01-1.97; P = 0.04], bioavailable estradiol (HR, 1.26; 95% CI, 1.03-1.53; P = 0.02), and free estradiol (HR, 1.31; 95% CI, 1.03-1.65; P = 0.03) were each associated with risk for recurrence. Thus, the risk of recurrence increased by 41% per unit increase in log estradiol. In this matched sample, women who recurred had an average total estradiol concentration that was double the average for the nonrecurred women (22.7 versus 10.8 pg/mL; P = 0.05). Likewise, average concentrations of free and bioavailable estradiol were more than double in the women who recurred compared with the nonrecurred women.

Controls had SHBG levels that were nonsignificantly higher and total, bioavailable, and free testosterone concentrations that were nonsignificantly lower than cases. SHBG and testosterone were not associated with risk for recurrence (Table 2). The same was true for the ratio of testosterone to estradiol using total, bioavailable, or free hormone fractions (data not shown).

Because time between diagnosis and study entry was marginally different between cases and controls, despite the fact that date of diagnosis and date of study entry were matching criteria in the case-control design, the analysis was rerun including time between diagnosis and study entry as a covariate. The resulting HRs for total,

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bioavailable, and free estradiol did not change substantially (<3%) from those reported in Table 2. Likewise, models adjusted for hot flash status, menopausal status, ethnicity, and dietary fiber intake showed only slight differences in HRs from those reported in Table 2. Similarly, adjustment for body weight and intervention group assignment (variables not included in Table 1) had a negligible effect on HRs. Higher bioavailable and free estradiol concentrations predicted recurrence in all models.

In women who reported hot flashes at study entry, average total estradiol concentration was lower than that in women who did not experience hot flashes (n = 199 versus 86, μ = 12.1 versus 24.9 pg/mL; P = 0.01). Similarly, average bioavailable estradiol (6.6 versus 13.7 pg/mL) and free estradiol (0.25 versus 0.51 pg/mL) concentrations were lower in the group who reported hot flashes (P = 0.01).

Discussion

Results from this study provide evidence that higher serum estrogen concentrations contribute to risk for recurrence in women who have been diagnosed and treated for early-stage breast cancer. In a nested case-control cohort of women who were matched on key factors such as cancer stage and age at diagnosis, average concentrations of total, bioavailable, and free estradiol were more than double in the women who recurred compared with the nonrecurred women and were independently directly associated with likelihood of recurrence. Given the proliferative effect of estrogens on human mammary cells and the hypothesized DNA-damaging effects of estrogen and related metabolites on these cells (1), this association between estrogen status and progression of breast cancer is consistent with expectations. Further, these results are consistent with a prior WHEL Study finding suggesting that the presence of hot flashes was associated with reduced risk for recurrence (25). In this sample, women reporting hot flashes had significantly lower serum concentrations of total, bioavailable, and free estradiol. Contrary to expectations, we did not observe a relationship between serum testosterone concentrations and risk for recurrence. Although SHBG concentrations defined the estrogen fractions that we examined, which allowed the demonstration that higher levels of fractions that can affect peripheral tissues were associated with recurrence, this hormonal factor was not independently associated with recurrence.

The relationship between estrogen and breast carcinogenesis is complex (1). Reproductive steroid hormones are biochemically related, so teasing out independent associations from a group of compounds that are readily interconverted may not be an appropriate goal. More importantly, genetic polymorphisms in the synthesis and metabolic pathways for steroid hormones likely influence the relationship between circulating hormone concentrations and actual tissue exposure and responsiveness. Further, the interpretation of observational data (epidemiologic or clinical) is constrained by the inherent limitations in these investigations, such as timing of blood collections, laboratory measurement capabilities, and influencing factors such as lifestyle factors and various treatment modalities.

Studies of serum reproductive hormones and risk for primary breast cancer in postmenopausal women have linked higher levels of estrogens and androgens, and lower level of SHBG (which determines the pool of estrogens that can enter cells), with increased risk for primary breast cancer (6). For example, in a pooled analysis of data from nine prospective studies involving 663 postmenopausal women who developed breast cancer and 1,765 women who did not, risk increased significantly with increasing concentrations of total, bioavailable, and free estradiol; estrone, estrone sulfate; and androstenedione, dehydroepiandrosterone, dehydroepiandrosterone sulfate, and testosterone (4). In that pooled analysis, the relative risks were at least 2-fold greater in the highest versus the lowest quintile for estradiol and testosterone concentrations, and a 34% increased risk was observed in the lowest versus highest quintile for SHBG. In participants in the National Surgical Adjuvant Breast and Bowel Project Cancer Prevention Trial, who are at particularly high risk of breast cancer, circulating levels of estradiol, testosterone, and SHBG were not predictive of risk for primary breast cancer or responsiveness to tamoxifen (28). That finding illustrates that other risk factors and physiologic, biochemical, and genetic factors that characterize the individual course of breast carcinogenesis and cellular activities can affect the effect of estrogen status on risk for breast cancer.

Data on the relationship between serum reproductive hormones and progression or survival following the diagnosis of breast cancer are limited. Serum concentrations of testosterone, estradiol, and SHBG and risk for recurrence were examined in 110 women who had been diagnosed and treated for breast cancer and had participated in the Diet and Androgens Trial-2 (13), another diet intervention study. Over a follow-up period of 5.5 years, 31 participants in that study experienced a new breast cancer event (contralateral breast cancer, distant metastasis, or local relapse). The HRs (95% CIs) were 1.8 (0.5-6.3) for the middle tertile and 7.2 (2.4-21.4) for the upper tertile of baseline testosterone concentration. Although serum concentration of estradiol also was higher in recurred women compared with women who did not recur, an independent effect was not observed when adjusted for testosterone levels. In 107 of those participants, an analysis of hormone levels measured at 1 year post-enrollment suggested that women who exhibited a reduction in testosterone concentration from above to below the median had a reduced risk of recurrence (HR, 0.15; 95% CI, 0.03-0.71) compared with women whose testosterone levels remained high at follow-up. In another more recent investigation (29), higher plasma testosterone concentration was observed to predict poorer prognosis in a cohort of 194 postmenopausal women diagnosed with early-stage breast cancer who participated in a randomized fenretinide trial as untreated controls. Notably, estrogen concentrations were not examined in that study. In contrast with these two small previous studies, testosterone concentrations were not significantly associated with recurrence-free survival in the present study, although the point estimates were lower in controls compared with cases. Differences in some characteristics of the study participants may explain the conflicting findings relating to testosterone and prognosis; for example, the women in the other more recent investigation never received chemotherapy or hormonal therapy (28).

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Genetic polymorphisms that affect synthesis and metabolism of reproductive steroid hormones would be expected to influence circulating levels of these hormones, peripheral tissue concentrations, and, potentially, risk for recurrence (1). In the Health, Eating, Activity and Lifestyle Study, associations between the *CYP17, COMT*, and *SHBG* polymorphisms and serum sex hormone concentrations were examined in 366 postmenopausal breast cancer survivors (27). In that study, no associations between any of the genotypes examined and sex hormone concentrations were observed when analyzing for main effects, although *CYP17* and *SHBG* variants were associated with a few differences in androgen, estradiol, and SHBG levels in small subsets of those participants. Whether these polymorphisms affect peripheral tissue concentrations or overall survival, within the context of potentially multiple polymorphisms and other influencing factors, such as diet, physical activity, treatment modalities, and other characteristics that contribute to risk, is unknown and will be challenging to disentangle.

As reported previously (15), the WHEL Study diet intervention (high in vegetables, fruit, and fiber and low in fat) did not have an overall effect on recurrence or survival. However, we have observed previously that the diet intervention was associated with an average 32% reduction in serum bioavailable estradiol concentration at 1 year following enrollment in a subset of study participants (19). That analysis, other populations (20), and laboratory animal studies (31) have identified dietary fiber as one dietary component that feasibly could modulate estrogen status by interfering with enterohepatic circulation. In the present analysis, we did not observe a difference in dietary fiber intake in women who recurred compared with women who did not recur.

This study has several limitations. The nested case-control design is a statistically powerful approach, and the number of participants in this study exceeds that examined in other analyses of serum reproductive hormone concentrations and risk for recurrence in breast cancer survivors; this is still a relatively small study, particularly in view of the heterogeneous nature of human breast carcinogenesis. Further, we recognize that numerous genetic and other factors, including diurnal variation, may influence serum hormone concentrations and their effect on risk for recurrence, which likely explains why a significant association with serum estrogen (and not testosterone or SHBG) concentration was identified in spite of using a powerful study design. Also, we examined blood samples from only one time point for this hormone analysis. In the general population, the within-person correlations between measurements of reproductive hormone concentrations collected at least 1 year apart are moderately high for estrogen and somewhat higher for testosterone and SHBG (6). However, women who have been diagnosed and treated for breast cancer may have greater variability in reproductive hormone concentrations, so characterizing status based on one measurement may be considered a limitation. The mean interval between diagnosis and WHEL Study enrollment was ~2 years (14, 15), and chemotherapy and tamoxifen usage was similar in the recurred and nonrecurred women in the cohort analyzed in the present study, so effects of treatment were unlikely to bias the analysis. Follow-up data on hormonal response to the diet intervention were also not available for this analysis, and we would expect those data, in addition to characterizing genetic polymorphisms, could provide additional insights.

In summary, this study found significant independent associations between serum concentrations of total, bioavailable, and free estradiol and risk for recurrence in a nested case-control study design involving 153 case-control pairs of women diagnosed and treated for early-stage breast cancer. Although genetic and metabolic factors likely modulate the relationship between circulating sex hormones and risk, results from this study provide evidence that higher serum estrogen concentration contributes to risk for recurrence in this population.

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