Elevated Serum Estradiol and Testosterone Concentrations Are Associated with a High Risk for Breast Cancer

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Background: The relation between endogenous steroid hormones and risk for breast cancer is uncertain. Measurement of sex hormone levels may identify women at high risk for breast cancer who should consider preventive therapies.

Objective: To test the hypothesis that serum concentrations of estradiol and testosterone predict risk for breast cancer.

Design: Prospective case–cohort study.

Setting: Four clinical centers in the United States.

Participants: 97 women with confirmed incident breast cancer and 244 randomly selected controls; all women were white, 65 years of age or older, and were not receiving estrogen.

Measurements: Sex-steroid hormone concentrations were assayed by using serum that was collected at baseline and stored at −190 °C. Risk factors for breast cancer were ascertained by questionnaire. Incident cases of breast cancer were confirmed by review of medical records during an average period of 3.2 years.

Results: The relative risk for breast cancer in women with the highest concentration of bioavailable estradiol (≥6.83 pmol/L or 1.9 pg/mL) was 3.6 (95% CI, 1.3 to 10.0) compared with women with the lowest concentration. The risk for breast cancer in women with the highest concentration of free testosterone compared with those with the lowest concentration was 3.3 (CI, 1.1 to 10.3). The estimated incidence of breast cancer per 1000 person-years was 0.4 (CI, 0.0 to 1.3) in women with the lowest levels of bioavailable estradiol and free testosterone compared with 6.5 (CI, 2.7 to 10.3) in women with the highest concentrations of these hormones. Traditional risk factors for breast cancer were similar in case-patients and controls. Adjustments for these risk factors had little effect on the results.

Conclusions: Estradiol and testosterone levels may play important roles in the development of breast cancer in older women. A single measurement of bioavailable estradiol and free testosterone may be used to estimate a woman's risk for breast cancer. Women identified as being at high risk for breast cancer as determined by these hormone levels may benefit from antiestrogen treatment for primary prevention.

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One in eight women in the United States will develop breast cancer, and deaths from breast cancer account for 17% of all cancer deaths in women in the United States (1, 2). In 1997, more than 180 000 new cases of breast cancer occurred in women in the United States (2); about half occurred in women 65 years of age or older. About 1 in 14 women aged 60 to 79 years will develop breast cancer compared with 1 in 26 women aged 40 to 59 years (2).

Endogenous estrogens may play an important role in the development of breast cancer (3). Some (4–8) but not all (9–12) prospective studies have found statistically significant positive associations between endogenous concentrations of estrogens and subsequent risk for breast cancer. Two recent reviews concluded that increasing evidence supports a relation between estrogen concentrations and risk for breast cancer (3, 13). Women with higher bone mineral density, which is a cumulative measure of endogenous estrogen, have an increased risk for breast cancer (14–16). Elevated endogenous serum concentrations of androgens may also be related to an increased risk for breast cancer (5, 7, 17), but this relation may not be independent of serum estrogens (8, 18, 19). The best estrogen fraction with which to predict risk has not been identified (3). Most studies have included measurements of total hormone levels; the concentrations of free hormones may have even stronger associations. Most of the women in these studies were postmenopausal and younger than 65 years of age.

Two randomized trials (20, 21) have shown a reduction in the occurrence of primary breast cancer in patients who have received tamoxifen and raloxifene. In the Breast Cancer Prevention Trial (20), 4 years of tamoxifen use led to a 45% reduction in breast cancer incidence in 13 388 women. Women in this study were considered to be at high risk for breast cancer on the basis of the presence of certain risk factors, including age of 60 years or older; about 30% of women were 60 years of age or older. The Multiple Outcomes of Raloxifene Evaluation (MORE) trial (21) found a 70% reduction in risk for breast cancer, especially cases of estrogen receptor–positive cancer, after 33 months of treat-

ment with raloxifene (21). About 80% of the 7704 women in this trial were older than 60 years of age.

Because treatment with tamoxifen or raloxifene entails costs and risk (20–22), it is important to identify women who are at greatest risk for breast cancer and are therefore most likely to benefit from antiestrogen therapies. Our study tests the hypothesis that serum concentrations of estradiol and testosterone, measured an average of 3 years before the clinical diagnosis of breast cancer, are related to risk for breast cancer in women 65 years of age or older. We hypothesized that measurements of serum hormones could be used to identify women at high risk for developing breast cancer. We used a case–cohort approach to compare serum hormone concentrations in 97 incident case-patients with breast cancer and 244 randomly selected controls.

Methods

Study Sample

All women were participants in the Study of Osteoporotic Fractures, a prospective study of 9704 white, community-dwelling women who were at least 65 years of age and were recruited at four clinical centers across the United States (23). Women were excluded from the study if they reported a bilateral hip replacement or the inability to walk without the assistance of another person. During 3.2 years of follow-up, we confirmed 121 cases of breast cancer, including 4 cases of carcinoma in situ, through review of medical records by a physician-epidemiologist (14). We excluded women who reported current estrogen replacement therapy at baseline; remaining were 97 confirmed cases of incident breast cancer. Using a case–cohort approach, we chose as controls a random sample of 247 women who survived to the first annual visit, denied a history of breast cancer, and did not report use of estrogen at baseline. Three of these women subsequently developed incident breast cancer and were included in the case-patient group. This study was approved by the biomedical institutional review board at each of the participating institutions. All participants provided informed consent.

Sex-Steroid Hormones

Serum specimens were obtained from all participants at a baseline examination in 1986 to 1988. All participants were instructed to adhere to a fat-free diet during the night and morning before the examination to minimize lipemia that might interfere with assays. Blood was drawn between 8:00 a.m. and 2:00 p.m., and serum was immediately frozen to −20 °C. Within 2 weeks, all samples were shipped to a central repository and stored in liquid nitrogen at −190 °C until the assays were performed. We measured total estradiol, bioavailable estradiol or estradiol that was not bound by sex hormone–binding globulin (SHBG), free estradiol, estrone, estrone sulfate, androstenedione, dehydroepiandrosterone sulfate, total and free testosterone, and SHBG. All assays were done by Corning Nichols Institute (San Juan Capistrano, California); researchers were blinded to participants' breast cancer status. The sensitivity of the assays refers to the lower limit of detection. Assays were performed concurrently on serum specimens from case-patients and controls.

The intra-assay and total assay variability is expressed as a coefficient of variation. In this study, a range of coefficient of variation includes values for a low-concentration quality-control sample to those of a high-concentration quality-control sample. When no range is reported, the coefficient of variation was similar for both low- and high-concentration quality-control samples.

Total estradiol was measured by using liquid–liquid organic extraction, column chromatography, and radioimmunoassay (coefficient of variation for intra-assay and total assay, 4% to 12% and 9% to 11%, respectively; sensitivity, 7.3 pmol/L). Free estradiol was measured by using equilibrium dialysis and calculated by using the percentage of dialyzable estradiol and total estradiol (coefficient of variation for intra-assay and total assay, 3% to 4% and 5%, respectively; sensitivity, 0.37 pmol/L). Percentage of non–SHBG-bound estradiol or bioavailable estradiol was measured by ammonium sulfate precipitation of SHBG-bound steroids (coefficient of variation for intra-assay and total assay, 3% and 6%, respectively). The amount of estradiol that was non–SHBG-bound was then calculated as the product of the total amount of estradiol and the percentage of nonbound estradiol.

Estrone was measured by using extraction, chromatography, and radioimmunoassay (coefficient of variation for intra-assay and total assay, 6% to 12% and 8% to 17%, respectively; sensitivity, 37 pmol/L). Estrone sulfate was measured by using organic extraction, enzymatic hydrolysis, celite chromatography, and radioimmunoassay (coefficient of variation for intra-assay and total assay, 6% to 7% and 7% to 8%, respectively; sensitivity, 143 pmol/L).

Androstenedione was measured by using a radioimmunoassay after preparation for analysis by organic extraction and chromatography (coefficient of variation for intra-assay and total assay, 6% to 10% and 7% to 16%, respectively; sensitivity, 0.10 nmol/L). Dehydroepiandrosterone sulfate was measured by using radioimmunoassay after preparation for the analysis by serial dilution (coefficient of varia-

tion for intra-assay and total assay, 6% to 11% and 9% to 12%, respectively; sensitivity, 0.17 μmol/L). Total testosterone was measured by using radioimmunoassay with chromatographic purification (coefficient of variation for intra-assay and total assay, 6% to 14% and 5% to 13%, respectively; sensitivity, 0.03 nmol/L). Free testosterone was measured by using equilibrium dialysis. Calculation of free testosterone was adjusted for albumin concentration (coefficient of variation for intra-assay and total assay, 5% and 5.4%, respectively; sensitivity, 34.7 pmol/L). Sex hormone–binding globulin was measured by using radioimmunoassay (coefficient of variation for intra-assay and total assay, 7% and 7.8%, respectively; sensitivity, 5.0 nmol/L).

We formed the ratio of estrone sulfate to estrone to test the hypothesis suggested by Dorgan and coworkers (5) that women who develop breast cancer may be less able than other women to metabolize estrone to a less active form.

We determined the reproducibility of selected hormone measurements in 20 postmenopausal women by assaying hormone levels in duplicate in different batches. Pearson correlations (all significant at $P < 0.001$) between the two measures were as follows: total testosterone, $r = 0.98$; free testosterone, $r = 0.97$; total estradiol, $r = 0.56$; non–SHBG-bound estradiol, $r = 0.83$; estrone, $r = 0.67$; estrone sulfate, $r = 0.70$; androstenedione, $r = 0.77$; dehydroepiandrosterone sulfate, $r = 0.97$; and SHBG, $r = 0.97$. Initial and repeated mean values were similar.

Variables

Weight (in lightweight clothing with shoes removed) was recorded with a balance-beam scale. Self-reported height at 25 years of age was used to calculate the modified body mass index because women with low bone mass experience height loss secondary to vertebral fractures. A reproductive history, obtained by questionnaire and interview, included information on ages at menarche, menopause, and first birth; parity; and family history of breast cancer. Participants were asked about past use of estrogen replacement therapy, current and lifetime use of cigarettes and alcohol, and whether they walked for exercise. We calculated the number of alcoholic drinks per week; nondrinkers were coded as having zero intake.

Statistical Analyses

Characteristics of case-patients and controls were compared by *t*-test (continuous variables) or by chi-square test (categorical variables). Sex-steroid hormone levels were not normally distributed. The nonparametric (Wilcoxon two-sample) test was used to compare the distribution of hormones in case-patients and controls.

For all hormones except free estradiol, the relative hazard (RH) for breast cancer was calculated (using the lowest quartile as the reference group) across quartiles of sex-steroid hormone levels by using a modification of the Cox proportional hazards model that accounted for the case–cohort sampling design; this modified model has been successfully applied in previous studies (25). Cut-points for quartiles were based on distribution within the random subset of the cohort. The distribution of free estradiol did not allow division by quartiles; four levels of free estradiol were assigned to approximate quartiles as closely as possible. A test was done for linear trend of increasing risk for breast cancer across quartiles of hormones.

We initially adjusted for age and modified body mass index. Multivariate models included adjustment for conventional risk factors for breast cancer, including age; modified body mass index; age at menarche, first birth, and menopause; surgical menopause (yes or no); nulliparity (yes or no); family history of breast cancer in a mother or sister (yes or no); past estrogen use (yes or no); walking for exercise (yes or no); and alcohol consumption. Unless otherwise noted, variables were entered as continuous variables. Alcoholic drinks were converted to grams per day, assuming an average of 11.5 grams per drink. Average number of grams consumed per day was categorized to conform with five categories (0 g/d, <1.5 g/d, 1.5 g/d to <5.0 g/d, 5.0 g/d to <15 g/d, and 15 or more g/d) that were typically used in other studies (25, 26). These categories were entered as dummy variables in the multivariate model.

In the Nurses' Health Study (8), the association between serum hormones and breast cancer was particularly strong in women who had never used estrogen. Therefore, we excluded past estrogen users in our study and redid our analyses.

We estimated the incidence of breast cancer per 1000 person-years and 95% CIs by levels of both bioavailable estradiol and free testosterone. For these analyses, we combined the two middle quartiles of hormones and calculated the incidence of breast cancer in relation to levels of bioavailable estradiol and free testosterone. To calculate incidence, we estimated total person-years within each category of bioavailable estradiol and free testosterone by applying the person-year distribution of the random sample of the cohort (controls) to the total number of person-years in the cohort. Rates were then obtained in the usual fashion by multiplying the ratio of the number of case-patients to the number of person-years by 1000 (to express in units per 1000 person-years). We estimated standard er-

Table 1. Descriptive Characteristics of Case-Patients and Controls*

Variable	Case-Patients (n = 97)	Controls (n = 244)	P Value
Age ± SD, *y*	70.9 ± 4.6	71.8 ± 5.0	0.14
Weight ± SD, *kg*	69.9 ± 13.1	67.7 ± 11.9	0.14
Body mass index ± SD, kg/m^2	27.6 ± 5.4	26.5 ± 4.3	0.07
Height at 25 years of age ± SD, *cm*	162.5 ± 6.2	163.2 ± 6.0	>0.2
Age at menarche ± SD, *y*	12.8 ± 1.6	13.1 ± 1.6	0.16
Age at first birth ± SD, *y*†	25.9 ± 5.5	25.3 ± 4.7	>0.2
Age at menopause ± SD, *y*	46.7 ± 5.5	47.6 ± 5.6	>0.2
Live births ± SD, *n*	2.48 ± 1.63	2.70 ± 1.48	>0.2
Surgical menopause, %	12.6	10.6	>0.2
Ever pregnant, %	84.5	79.1	>0.2
Nulliparous, %	17.2	21.2	>0.2
Family history of breast cancer, %	14.7	14.2	>0.2
Walks for exercise, %	54.6	52.5	>0.2
Current smoker, %	5.3	8.2	>0.2
Drank alcohol within the past 12 months, %	74.2	70.1	>0.2
Median drinks per week (range), *n*	0.63 (0–22)	0.49 (0–21)	0.03
Past estrogen use, %	33.7	32.0	>0.2
Time elapsed since stopping estrogen ± SD, *y*‡	12.4 ± 8.5	6.1 ± 8.6	>0.2
Duration of estrogen use ± SD, *y*‡	5.8 ± 6.2	6.5 ± 7.1	>0.2

* Values expressed with SDs are means.
† Among parous women.
‡ Estrogen users only.

rors by assuming a Poisson distribution for occurrence of events and by using a Taylor expansion to account for the additional variability introduced by the estimation of person-years.

To test the hypothesis that the association between breast cancer and the precursor hormone (androstenedione or dehydroepiandrosterone sulfate) could be explained by levels of bioavailable estradiol and free testosterone, we calculated the RH for breast cancer in multivariate models that included all three hormones: androstenedione (or dehydroepiandrosterone sulfate), bioavailable estradiol, and free testosterone. For these analyses, we dichotomized the hormone variables and compared women in the top three quartiles with those in the lowest quartile. Adjustment for age and body mass index was included in these models.

Role of the Funding Source

The funding sources did not participate in the design and conduct of the study or reporting of results and had no role in the decision to submit this paper for publication.

Results

Case-patients and controls (random sample of the cohort) were similar with respect to age, reproductive history, family history of breast cancer, smoking, exercise, and other conventional risk factors for breast cancer (**Table 1**). The mean body weight and body mass index tended to be higher in case-patients. Case-patients reported more consumption of alcohol in the past year. About one third of case-patients and one third of controls reported past use of estrogen replacement therapy. Case-patients and controls did not differ significantly in the number of years since discontinuing use of estrogen or in duration of estrogen use.

Sex-Steroid Hormones and Breast Cancer

Median concentrations of sex-steroid hormone were higher in case-patients than in controls (**Table 2**). In particular, total estradiol and bioavailable estradiol concentrations were about 30% higher and free testosterone concentrations were 28% higher. Case-patients and controls differed significantly in distribution of all hormones except SHBG.

The association between serum hormone level and breast cancer was strongest for bioavailable estradiol: Women in the highest quartile of estradiol concentration had a 3.6-fold greater risk for breast cancer than women in the lowest quartile of estradiol concentration (95% CI, 1.3-fold to 10.0-fold) (**Table 3**). Among the androgens, total and free testosterone concentrations were strongly linked to subsequent risk for breast cancer; risk was three times greater in women with the highest concentrations of testosterone. These associations were independent of age, body mass index, and other conventional risk factors for breast cancer.

Women in the highest quartile of estrone, estrone sulfate, androstenedione, and dehydroepiandrosterone sulfate concentrations also had an in-

Table 2. Median and Range of Concentrations of Sex-Steroid Hormones in Case-Patients and Controls*

Sex-Steroid Hormones	Median for Case-Patients (Range)	Median for Controls (Range)	P Value†
Estrogens			
Estradiol, *pmol/L*	29.4 (11–81)	22.0 (7–206)	<0.001
Non–SHBG-bound estradiol, *pmol/L*	4.8 (1.10–23.9)	3.7 (0.70–38.9)	0.001
Free estradiol, *pmol/L*	0.5 (0–1.5)	0.44 (0–4.4)	<0.001
Estrone, *pmol/L*	88.8 (0–255.2)	74.0 (0–248.0)	0.004
Estrone sulfate, *pmol/L*	630.7 (120–2957)	459.5 (0–3108)	0.004
Androgens			
Androstenedione, *nmol/L*	1.54 (0.17–5.31)	1.26 (0–5.13)	0.003
Dehydroepiandrosterone sulfate, *μmol/L*	2.04 (0.24–9.69)	1.72 (0–9.04)	0.04
Total testosterone, *nmol/L*	0.73 (0–2.70)	0.62 (0–2.63)	0.005
Free testosterone, *pmol/L*	10.4 (0–38.8)	8.1 (0–34.3)	0.003
SHBG, *nmol/L*	38.0 (6–89)	43.0 (5–119)	0.2

* SHBG = sex hormone–binding globulin.
† Wilcoxon two-sample test.

Table 3. Relative Hazard for Breast Cancer by Concentration of Sex-Steroid Hormones*

Sex-Steroid Hormones	Case-Patients	Controls	Relative Hazard Adjusted for Age	Relative Hazard (95% CI) Adjusted for Age and BMI	*P* Value for Trend	Relative Hazard Adjusted for Conventional Breast Cancer Risk Factors (95% CI)†	*P* Value for Trend
	n						
Total estradiol							
Level 1 (<18.4 pmol/L)	14	60	1.0	1.0 (referent)	0.018	1.0 (referent)	0.061
Level 2 (18.4–<22.0 pmol/L)	15	39	1.8	1.8 (0.7–4.2)		1.9 (0.7–5.2)	
Level 3 (22.0–<29.4 pmol/L)	17	66	1.0	1.0 (0.4–2.3)		0.9 (0.3–2.3)	
Level 4 (≥29.4 pmol/L)	51	78	2.8	2.7 (1.3–5.8)		2.9 (1.2–7.2)	
Bioavailable estradiol							
Quartile 1 (<2.20 pmol/L)	10	60	1.0	1.0 (referent)	0.034	1.0 (referent)	0.063
Quartile 2 (2.2–<3.78 pmol/L)	30	61	2.9	3.0 (1.3–6.8)		3.5 (1.2–10.8)	
Quartile 3 (3.78–<6.83 pmol/L)	21	60	1.9	1.8 (0.8–4.4)		2.2 (0.8–6.6)	
Quartile 4 (≥6.83 pmol/L)	36	61	3.6	3.4 (1.4–8.3)		3.6 (1.3–10.0)	
Free estradiol							
Level 1 (<0.37 pmol/L)	4	25	1.0	1.0 (referent)	0.021	1.0 (referent)	0.032
Level 2 (0.37–<0.73 pmol/L)	58	170	1.7	1.7 (0.6–5.3)		1.6 (0.4–6.7)	
Level 3 (0.73–<1.10 pmol/L)	25	32	4.5	4.7 (1.3–16.7)		4.8 (0.9–25.4)	
Level 4 (≥1.10 pmol/L)	10	16	2.8	2.9 (0.7–12.1)		3.1 (0.5–20.2)	
Estrone							
Quartile 1 (<51.8 pmol/L)	14	53	1.0	1.0 (referent)	0.036	1.0 (referent)	0.108
Quartile 2 (51.8–<74.0 pmol/L)	21	67	1.3	1.3 (0.6–2.8)		0.9 (0.3–2.4)	
Quartile 3 (74.0–<103.6 pmol/L)	29	62	1.7	1.7 (0.8–3.6)		1.5 (0.6–3.9)	
Quartile 4 (≥103.6 pmol/L)	33	61	2.4	2.3 (1.0–5.2)		1.8 (0.6–5.1)	
Estrone sulfate							
Quartile 1 (<305.4 pmol/L)	17	61	1.0	1.0 (referent)	0.041	1.0 (referent)	0.141
Quartile 2 (305.4–<476.6 pmol/L)	18	62	1.1	1.0 (0.5–2.3)		0.8 (0.3–2.1)	
Quartile 3 (476.6–<756.3 pmol/L)	26	60	1.5	1.4 (0.7–3.0)		1.2 (0.4–3.3)	
Quartile 4 (≥756.3 pmol/L)	36	60	2.1	2.0 (0.9–4.2)		1.8 (0.7–4.7)	
Androstenedione							
Quartile 1 (<0.84 nmol/L)	14	60	1.0	1.0 (referent)	0.017	1.0 (referent)	0.026
Quartile 2 (0.84–<1.26 nmol/L)	19	59	1.4	1.3 (0.6–3.0)		1.2 (0.5–3.4)	
Quartile 3 (1.26–<1.78 nmol/L)	25	60	1.5	1.4 (0.7–3.1)		1.3 (0.5–3.6)	
Quartile 4 (≥1.78 nmol/L)	39	65	2.4	2.4 (1.2–4.9)		2.9 (1.1–7.9)	
Dehydroepiandrosterone sulfate							
Quartile 1 (<1.00 μmol/L)	16	58	1.0	1.0 (referent)	0.040	1.0 (referent)	0.027
Quartile 2 (1.00–<1.74 μmol/L)	25	64	1.3	1.2 (0.6–2.6)		1.0 (0.4–2.7)	
Quartile 3 (1.74–<2.71 μmol/L)	23	61	1.5	1.5 (0.7–3.1)		1.3 (0.5–3.7)	
Quartile 4 (≥2.71 μmol/L)	33	61	2.2	2.1 (1.0–4.4)		2.4 (0.9–6.3)	
Total testosterone							
Quartile 1 (<0.42 nmol/L)	10	57	1.0	1.0 (referent)	0.010	1.0 (referent)	0.008
Quartile 2 (0.42–<0.62 nmol/L)	25	61	2.2	2.1 (0.9–5.0)		2.2 (0.7–7.1)	
Quartile 3 (0.62–<0.97 nmol/L)	30	66	3.0	3.0 (1.3–6.7)		5.5 (1.8–17.0)	
Quartile 4 (≥0.97 nmol/L)	32	60	2.9	2.8 (1.2–6.5)		3.6 (1.1–11.7)	
Free testosterone							
Quartile 1 (<5.54 pmol/L)	10	56	1.0	1.0 (referent)	0.010	1.0 (referent)	0.009
Quartile 2 (5.54–<8.32 pmol/L)	23	65	1.8	1.7 (0.7–4.2)		2.2 (0.6–7.5)	
Quartile 3 (8.32–<13.17 pmol/L)	33	58	3.7	3.5 (1.5–8.2)		6.4 (2.1–19.6)	
Quartile 4 (≥13.17 pmol/L)	31	64	2.7	2.5 (1.1–6.0)		3.3 (1.1–10.3)	
Sex hormone–binding globulin							
Quartile 1 (<29 nmol/L)	22	58	1.0	1.0 (referent)	>0.2	1.0 (referent)	>0.2
Quartile 2 (29–<43 nmol/L)	36	60	1.5	1.5 (0.7–3.0)		1.4 (0.6–3.4)	
Quartile 3 (43–<59 nmol/L)	23	62	0.9	1.0 (0.5–2.2)		1.4 (0.5–3.8)	
Quartile 4 (≥59 nmol/L)	16	64	0.6	0.7 (0.3–1.6)		0.5 (0.2–1.7)	
Ratio of estrone sulfate to estrone							
Quartile 1 (<6.13)	16	61	1.0	1.0 (referent)	>0.2	1.0 (referent)	>0.2
Quartile 2 (6.13–<9.00)	30	61	1.7	1.7 (0.8–3.5)		2.2 (0.8–5.6)	
Quartile 3 (9.00–13.10)	29	60	1.8	1.8 (0.9–3.7)		2.2 (0.9–5.7)	
Quartile 4 (≥13.10)	22	61	1.3	1.3 (0.6–2.8)		1.1 (0.4–3.2)	

* BMI = body mass index.
† Adjusted for age; body mass index; ages at menarche, first birth, and menopause; nulliparity; family history of breast cancer; physical activity; surgical menopause; and alcohol consumption.

creased risk for breast cancer (**Table 3**). Sex hormone–binding globulin and the ratio of estrone sulfate to estrone were not associated with breast cancer. Results were similar when we excluded women who had used estrogen in the past.

The estimated incidence of breast cancer was lowest (0.4 per 1000 person-years [CI, 0 to 1.29]) in women with the lowest levels of bioavailable estradiol and free testosterone (**Figure**). In contrast, the incidence of breast cancer was 6.5 per 1000 person-years (CI, 2.7 to 10.3) in women with the highest concentration of both hormones.

Precursor Hormones

We tested the hypothesis that the precursor hormones, androstenedione or dehydroepiandrosterone

sulfate, were not independently related to breast cancer. In a model that included levels of bioavailable estradiol, free testosterone, and androstenedione, bioavailable estradiol (RH, 2.5 [CI, 1.2 to 5.3]) was independently related to breast cancer. A twofold increased risk for breast cancer was related to the level of free testosterone, but the CI included 1.0 (RH, 2.1 [CI, 0.9 to 4.7]). Androstenedione was not related to the risk for breast cancer (RH, 1.2 [CI, 0.6 to 2.4]). Similar results were obtained in models that used bioavailable estradiol (RH, 2.5 [CI, 1.2 to 5.3]), free testosterone (RH, 2.1 [CI, 0.9 to 4.6]), and dehydroepiandrosterone sulfate (RH, 1.2 [CI, 0.6 to 2.3]). Inclusion of androstenedione and dehydroepiandrosterone sulfate in the same model yielded similar results.

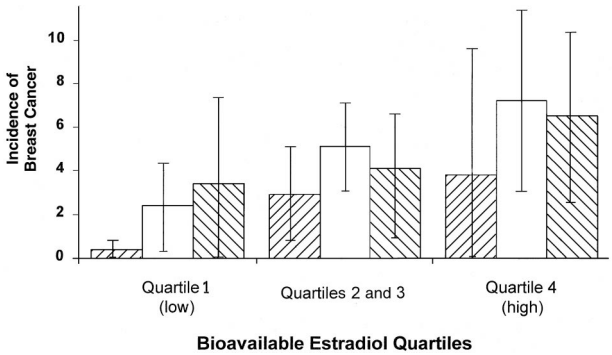

Figure. Incidence of breast cancer (95% CI) in relation to concentrations of bioavailable estradiol and free testosterone. Incidence expressed per 1000 person-years. Quartile 1: free testosterone, up-slanting diagonally striped bar; quartiles 2 and 3: free testosterone, white bar; quartile 4: free testosterone, down-slanting diagonally striped bar.

Discussion

The results of this study support the hypothesis that sex hormones are an important factor in the development of breast cancer in older women. In particular, women with a bioavailable estradiol concentration greater than 7 pmol/L (1.9 pg/mL) had a risk for breast cancer that was 3.6-fold greater than that in women with the lowest concentration of bioavailable estradiol. We also found a strong relation between the unbound portion of testosterone and the risk for breast cancer. Our results are consistent with those of other prospective studies of the relation between sex-steroid hormone levels and the risk for breast cancer in somewhat younger women (4, 5, 8).

The average incidence of breast cancer in white women 65 years of age and older in the United States is 4.6 per 1000 person-years (27). On the basis of our results, we estimate that the incidence of breast cancer in women with the highest concentrations of bioavailable estradiol and free testosterone is about 40% higher than this expected rate. The magnitude of the relative risk is similar to that of the strongest risk factors for breast cancer (personal history of ductal carcinoma in situ and atypical hyperplasia) (28).

The absolute concentrations of hormones, especially estradiol, were very low but are consistent with those previously reported in postmenopausal women (8). Nonetheless, a gradient of risk was observed across increasing concentrations. This gradient of risk is greater than that observed between serum cholesterol concentrations and coronary heart disease, especially in older women (3). Our results suggest that measurement of bioavailable estradiol and free testosterone may be used as a clinical measure to identify women at high-risk for breast cancer who may benefit from antiestrogen treatment. Clinical trials to test the effects of antiestrogen therapies on breast cancer risk related to estrogen concentrations are ongoing.

Our results also suggest that interventions to reduce serum hormone concentrations may reduce risk for breast cancer. A low-fat diet (29–31), weight reduction (32), and a vegetarian diet (33) have been shown to reduce levels of sex-steroid hormones. We previously reported an inverse association between concentrations of serum estrone and physical activity (34). In the Women's Health Trial (31), a 10- to 22-week low-fat diet intervention was associated with a 17% reduction in estradiol concentrations in healthy postmenopausal women. The Women's Health Initiative (35) is directly testing whether low-fat dietary patterns will reduce the incidence of breast cancer.

Sources of testosterone in postmenopausal women include direct secretion from the ovary and from the precursor hormones, androstenedione or dehydroepiandrosterone sulfate. Testosterone could influence the risk for breast cancer directly or indirectly (as a source of estradiol). Androgen receptors have been identified in human breast cancer cells, although in vitro activation of the androgen receptor tends to suppress the proliferation of breast cancer cells (36). In three studies, the association between levels of total testosterone and breast cancer was not independent of levels of bioavailable estradiol (8, 18, 19). However, these studies did not measure concentrations of free testosterone. In our study, we found an association of free testosterone levels to breast cancer that was independent of bioavailable estradiol levels, thereby suggesting a direct association.

The primary source of estrogens in postmenopausal women is the aromatization of androstenedione, an adrenal hormone (37). We and others (5) found an association between breast cancer and higher concentrations of androstenedione and dehy-

droepiandrosterone sulfate. However, in our study, the association between breast cancer and concentrations of androstenedione and dehydroepiandrosterone sulfate was no longer significant in models that included concentrations of bioavailable estradiol and free testosterone; this finding is consistent with the hypothesis that increased concentrations of androstenedione or dehydroepiandrosterone sulfate, as a precursor to estradiol and testosterone, may contribute to the increased risk for breast cancer.

Local formation of androgens and estrogens in the breast may also contribute to the development of breast cancer. Breast fat has aromatase activity, and levels of aromatase activity in adipose tissue adjacent to malignant tumors were significantly higher than those in tissues adjacent to benign lesions in one study (38). Breast tissue also contains a sulfatase enzyme that can convert estrone sulfate to estrone, which can then be converted to estradiol, thereby increasing the level of estradiol in the breast (39). In our study, both estrone and estrone sulfate were directly related to risk for breast cancer. Additional enzymatic processes, including the 17β-estradiol dehydrogenase, could allow high levels of sex-steroid hormones to accumulate in breast tissue (40, 41). It is unlikely, however, that an increase in estrogen synthesis in the breast could account for the increased blood levels of estradiol that we observed in women with breast cancer.

Breast cancer generally requires several years to become clinically or radiographically detectable (42). Breast tumor aromatase activity may be more important than aromatase in breast fatty tissue for the maintenance of tumor estradiol levels (43). Thus, we cannot exclude the possibility that higher levels of serum estrogens in case-patients reflect enhanced production of estrogen within the tumor itself. However, in the Nurses' Health Study (8), exclusion of case-patients who had received a diagnosis of breast cancer within 1 year of initial blood collection had no effect on the results. Longer follow-up will be needed to evaluate the long-term relation between serum hormones and breast cancer.

Weight gain, obesity, and increased intra-abdominal fat have all been identified as possible risk factors for breast cancer (44, 45), possibly because of aromatization of androstenedione to estrone in fatty tissue (46). In our study, adjustment for obesity as measured by body weight or body mass index did not substantially influence the association between sex-hormone concentration and breast cancer. However, we did not measure thigh fat mass, which has been associated with greater aromatase activity and therefore higher blood levels of estrone and estradiol (47), or intra-abdominal fat mass, which has been associated with greater concentrations of insulin, free and bioavailable estradiol, and testosterone (46, 47). Future studies should include these measures.

Measures of traditional risk factors for breast cancer, such as age at first birth, nulliparity, early menarche, and family history of breast cancer, were remarkably similar between case-patients and controls; our findings suggest that these conventional risk factors cannot accurately identify older women at high risk for breast cancer. Our results are consistent with those of other studies of older women (8). In addition, these risk factors are highly prevalent. In one study, more than 98% of the population had at least one of these risk factors (48), but most women with one or more of these risk factors do not develop breast cancer. Hence, it is unlikely that they can be used to identify older women at risk for breast cancer.

Our study has several limitations. Our cohort consists primarily of healthy, community-dwelling elderly white women; however, the overall rate of breast cancer in our cohort (4.3 per 1000 person-years) was similar to that observed for white women aged 65 years and older in the United States (27). Our results may not apply to women of other ethnic groups. The concentrations of hormones in these elderly women are relatively low and may be subject to increased laboratory variability. However, when the same laboratory was used, the reproducibility of sex-steroid hormone concentrations in postmenopausal women was excellent (49). Hormone concentrations were measured only once, and a single measure is always imprecise to some degree. We used a specialized endocrine laboratory; however, laboratory methods must be standardized before routine clinical laboratories are used to screen women for risk for breast cancer in relation to serum hormone concentrations. Although this is one of the largest cohort studies of breast cancer, we had limited power to test for interactions among hormones and breast cancer. Current hormone levels may not reflect earlier levels. However, several studies have documented correlations of serum estrogens over several years, especially in women whose weight remains stable (49–51). Thus, it is possible that the levels of hormones measured in these women may reflect exposures over a longer period.

Estradiol and testosterone play important roles in the risk for breast cancer in older women. Concentrations of these hormones predict the risk for breast cancer and may help clinicians decide about treatments to decrease breast cancer risk.

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