Serum Sex Hormone Levels After Menopause and Subsequent Breast Cancer

*Franco Berrino, Paola Muti, Andrea Micheli, Gianfranco Bolelli, Vittorio Krogh, Raffaella Sciajno, Paola Pisani, Salvatore Panico, Giorgio Secreto**

Background: **High levels of androgens and estrogens have been reported to be associated with breast cancer. However, the multiplicity of factors that influence hormone levels and methodologic issues complicate the study of the relationship between steroid sex hormones and breast cancer.** ***Purpose:*** **Using an improved study design, we assessed prospectively the relationship between the principal steroid sex hormones in serum and the subsequent occurrence of invasive breast cancer in postmenopausal women.** ***Methods:*** **Four thousand fifty-three healthy postmenopausal women, aged 40-69 years, were enrolled from June 1987 through June 1992 in a prospective investigation of hormones and diet in the etiology of breast tumors (ORDET study) as part of a larger volunteer cohort of 10 788 premenopausal and postmenopausal women from Varese Province, northern Italy. At recruitment, blood samples were taken between 8:00 AM and 9:30 AM (after overnight fasting), and sera were preserved in −80 °C freezers. Women who had received hormone treatment in the 3 months prior to enrollment, who had a bilateral ovariectomy, or who had a history of cancer or liver disease were not recruited. Twenty-five women in the final eligible cohort of 4040 postmenopausal women developed histologically confirmed, invasive breast cancer during the first 3.5 years of follow-up for the cohort (13 537 woman-years). For each case subject, four control subjects were randomly chosen after matching for factors possibly affecting hormone preservation in serum. One case subject and eight control subjects were excluded because premenopausal hormonal patterns were found; thus, after also excluding the four control subjects matched to the ineligible case subject, we included 24 case and 88 control subjects. In the spring of 1994, stored sera of case and control subjects were assayed in a blinded manner for dehydroepiandrosterone sulfate and estradiol (E_2) by in-house radioimmunoassay and for total and free testosterone and sex hormone-binding globulin by commercially available nonextraction iodination kits. Mean differences in risk factors were tested by analysis of variance for paired data. Relative risks (RRs) were estimated by conditional logistic regression analysis. All *P* values resulted from two-sided tests.** ***Results:*** **Age-adjusted mean values of total testosterone, free testosterone, and E_2 were significantly higher in case subjects than in control subjects: total testosterone, 0.34 ng/mL versus 0.25 ng/mL (*P*<.001); free testosterone, 1.07 pg/mL versus 0.77 pg/mL (*P* = .006); and E_2, 25 pg/mL versus 22 pg/mL (*P* = .027). Age-adjusted RRs for breast cancer in increasing tertiles were as follows: for total testosterone, 1.0, 4.8, and 7.0 (*P* for trend = .026); for free testosterone, 1.0, 1.8, and 5.7 (*P* for trend = .005); and for total E_2, 1.0, 7.1, and 5.5 (*P* for trend = .128).** ***Conclusions and Implications:*** **This prospective study provides further evidence in support of the already established association between elevated estrogen levels and breast cancer. Even more importantly, it provides new evidence that high serum testosterone levels precede breast cancer occurrence. [J Natl Cancer Inst 1996;88:291-6]**

The risk of developing breast cancer is related to events of reproductive life. This and many other characteristics of the disease suggest that endogenous steroid sex hormones play a role in the carcinogenic process, and a number of etiologic hypotheses involving these hormones have been proposed (*1,2*).

Attempts have been made to elucidate the role of sex hormones by measuring their levels in the blood and urine of breast cancer patients and healthy control subjects (*2,3*); these attempts included cohort studies in which blood or urine samples were collected before breast cancer diagnosis and stored for some years (*4-8*). Estrogens have been studied extensively, but so too have adrenal androgens (mainly dehydroepiandrosterone and dehydroepiandrosterone sulfate [DHEAS]), ovarian androgens (mainly testosterone), and free sex hormones, i.e., the fraction of estradiol (E_2) and testosterone not bound to sex hormone-binding globulin (SHBG) or other plasma proteins. Free sex hormones are considered to be the most active fraction at target organs.

Although cohort and other studies generally point to an association between levels of sex hormones and breast cancer, they have produced contradictory results (*3*); studies on hormones and breast cancer, however, have frequently been small and may have serious methodological limitations (*3*). Recently, a clear association between breast cancer risk and serum levels of circulating estrogens has been shown in a well-designed, large, prospective epidemiologic study carried out in New York City (*8*).

The present report addresses the relationship between invasive breast cancer and prediagnostic serum levels of the main androgens (i.e., DHEAS, total testosterone, and free testosterone) and of total E_2 and SHBG in postmenopausal women enrolled in the prospective cohort ORDET (hormones and diet in the etiology of breast tumors) study (*9*). The ORDET study was designed to control for or eliminate several sources of bias and undesired variability that have probably blurred the results of some previous studies.

**Affiliations of authors:* F. Berrino, P. Muti, A. Michell, V. Krogh, G. Secreto, Istituto Nazionale Tumori, Milan, Italy; G. Bolelli, Istituto di Clinica Ostetrica a Ginecologica "P Sfameni," Università di Bologna, Italy; R. Sciajno, Istituto Nazionale Tumori, Milan, and Istituto di Clinica Ostetrica e Ginecologica "P Sfameni," Università di Bologna; P. Pisani, Unit of Descriptive Epidemiology, International Agency for Research on Cancer, Lyon, France; S. Panico, Istituto di Medicina Interna e Malattie Dismetaboliche, Facoltà di Medicina, Università Federico II, Naples, Italy.

Correspondence to: Franco Berrino, M.D., Divisione di Epidemiologia, Istituto Nazionale per lo Studio a la Cura dei Tumori, Via Venezian, 1, 20133 Milan, Italy.

See "Notes" section following "References."

Subjects and Methods

From June 1987 to June 1992, 10 788 women aged 35-69 years, residing in Varese Province, northern Italy, an area covered by the Lombardy Cancer Registry (*10*), volunteered to be enrolled in the study. Women undergoing hormone treatment in the 3 months prior to admission, those with ongoing chronic or acute liver diseases, or those with a history of cancer or bilateral ovariectomy were not recruited. A total of 4053 women were recruited after menopause (provisionally defined as absence of menses for at least the 12 months before enrollment). We obtained signed informed consent forms from all participants. The Ethical Review Board of the National Cancer Institute, Milan, approved the study.

The ORDET procedures for gathering information on the enrolled women were described elsewhere (*9*). Trained nurses acquired data on menstrual and reproductive histories and other potential risk factors for breast cancer according to standardized procedures, adherence to which was periodically checked. Anthropometric measurements were taken of the women, who were in light clothes and without shoes at the time of the measurements. The repeatability of common risk factor data was checked on 233 cohort members a year after enrollment. The resulting correlation coefficients (Pearson's *r*) were .90 for age at menarche, .86 for age at menopause, .98 for number of births, .99 for age at first birth, .95 for weight, .98 for height, and .91 for waist and hip circumferences.

Blood Sampling and Serum Storage

At the time of recruitment, antecubital venous blood (40 mL) was drawn between 8:00 AM and 9:30 AM from subjects who had fasted overnight and had drunk one glass of water at about 7:30 AM. The exact time of venipuncture was recorded. Women were enrolled and blood samples were taken at two centers: in the hospital where the blood was processed and in another hospital 20 km away. Samples from the former hospital were processed by 11:00 AM; those from the latter hospital arrived daily and were processed by 1:00 PM. The sera were stored at 4 °C until about 5:30 PM, at which time they were placed in freezers for long-term storage. The temperature of the freezer was monitored continuously. No thawing accidents or significant warming occurred. However, the temperature ranged from −80 °C at the bottom of the freezer to about −50 °C at the top. Long-term cryoconservation of serum hormones in the ORDET freezers is being monitored in an ongoing ancillary study: After 3 years of storage, the levels of total E_2 and total testosterone proved to be rather stable, but free testosterone levels increased significantly (*11*).

For the assessment of intra-individual variability of hormone levels with time, 50 postmenopausal participants gave a second blood sample a year after giving the first; these two samples were assayed together for some hormones. The intraclass correlation coefficients were as follows: .71 for free testosterone, .87 for total testosterone, and .90 for DHEAS.

Breast Cancer Case Subjects

The 4053 women were followed to June 30, 1993, through the cancer registry and the regional and municipal files on residents; the mean time of follow-up was 3.5 years (range, 1-6 years). Eleven women were excluded from the cohort because linkage revealed that they had cancer prior to the enrollment date, and two more were excluded because they were lost to follow-up. The final postmenopausal cohort consisted of 4040 women, who were followed for 13 537 woman-years. Linkage identified 25 invasive breast cancer cases (27 were expected from Varese Province incidence rates). All cases were histologically confirmed. Four women with noninvasive breast cancer were not included.

Control Subjects

For each breast cancer case subject, four matched control subjects were randomly chosen from women in the cohort with the same menopausal status (as provisionally defined at recruitment) who were alive and free of breast cancer at the time of the breast cancer diagnosis. The control subjects were matched for the following criteria: (*a*) same recruitment center (to exclude differences due to transport of samples to laboratory), (*b*) recruited ±89 days from recruitment date of the breast cancer case (to allow for the effect of long-term preservation of sera), (*c*) recruited within the same daylight saving period (to allow for possible changes in circadian rhythm), and (*d*) serum samples stored in the same freezer and at the same level within the freezer (bottom, middle, or top).

Analysis of Serum Samples

Stored serum samples from case and control subjects were handled identically and assayed together in the same batch in the spring of 1994. All samples were assayed in duplicate. Each batch included samples from five to seven case subjects, from their matched control subjects, and from at least three commercial lyophilized sera (Lyphochek; Bio-Rad, Milan, Italy) inserted at the beginning, middle, and end of each batch. All laboratory personnel were blinded with regard to subject status (case versus control).

Total testosterone was assayed with a nonextraction double-antibody radioimmunoassay (RIA) kit from Sorin Biomedica, Saluggia, Italy. Free testosterone was measured by coated-tube RIA with a kit from Diagnostic Products Corporation (Medical Systems, Genoa, Italy). SHBG was measured by immunoradiometric assay from Radim-Techland S.A, Pomezia, Italy. DHEAS and total E_2 were measured by use of in-house RIA using tritiated tracers, dextran-coated charcoal (for separation of bound and free hormone), and highly specific antisera (*12*). Specifically, DHEAS was assayed directly using diluted serum according to the procedure of Buster and Abraham (*13*). Total E_2 was assayed after extraction because direct methods are unreliable at the low concentrations found in postmenopausal women. Isolute C18 cartridges (Stepbio, Bologna, Italy) were used in the extraction, as reported (*14*), and the assay was performed directly on the solvent extract without further purification. We monitored extraction efficiency by spiking all samples with a radioactive tracer; recovery was 74% ± 4% (mean ± standard deviation; based on 374 analytic determinations). Reported values were corrected for these losses. Anti-DHEA-3-succinyl bovine serum albumin had 0.4% and less than 0.1% cross-reactivity for androsterone and etiocholanolone sulfates, respectively; anti-estradiol-6-carboxymethyloxime bovine serum albumin cross-reactivity was 1.6% and 0.6% for estrone and estriol, respectively.

The intra-assay and inter-assay coefficients of variation were, respectively, 9.5% (based on 54 analytical determinations on lyophilized sera) and 7.3% (based on n = 9 assays) for total testosterone at 0.78 ng/mL, 13.5% (n = 45 assays) and 15.2% (n = 9 assays) at 1.82 pg/mL for free testosterone, 7.3% (n = 36 assays) and 9.6% (n = 9 assays) at a level of 67 pg/mL for total E_2, 9.5% (n = 35 assays) and 3.7% (n = 9 assays) at 4.62 μg/mL for DHEAS, and 4.1% (n = 27 assays) and 4.4% (n = 4 assays) at 71.9 nmol/L for SHBG.

Data Analysis

One case and eight control subjects were excluded from the postmenopausal group because premenopausal hormone patterns were found (follicle-stimulating hormone [FSH] levels <30 mIU/mL; ratio of FSH to luteinizing hormone ≤2.5); hysterectomy several years before recruitment had determined postmenopausal classification in six of these control subjects. The four control subjects matched to the misclassified breast cancer case subject were also excluded. Twenty-four case subjects and 88 control subjects were eventually available for analysis.

Age-adjusted means of serum hormone levels and other risk factors for breast cancer in case and control subjects were computed, and the mean of the differences between case subjects and matched control subjects was tested by multivariate analysis of variance for paired data. In testing, to allow for the non-normal distribution of values found for free testosterone, total testosterone, and E_2, we applied logarithmic transformations.

Study subjects were then divided into three groups according to the tertiles of the distribution of concentration of each hormone in control subjects. Using the lowest group as reference, we computed odds ratio estimates of relative risks (RRs). Matching variables and potential confounding factors were simultaneously accounted for by conditional logistic regression analysis. Age was treated as a continuous variable. In the computation of the *P* value for trend of linearity, hormones and SHBG were also kept as continuous variables. All *P* values resulted from two-sided statistical tests.

Results

The characteristics of the breast cancer case and control subjects with respect to known risk factors for breast cancer are summarized in Table 1. Because case subjects were older than control subjects (mean age of 59.4 years [range, 51-68 years] for case subjects versus mean age of 54.9 years [range, 40-69 years] for control subjects), both crude and age-adjusted means are given; however, both differed from each other only slightly. Case subjects had slightly higher weight

Table 1. Mean values of common risk factors for breast cancer in 24* postmenopausal case subjects and 88 matched control subjects from the ORDET cohort

	Crude mean ± standard deviation			Age-adjusted mean		
	Case subjects	Control subjects	*P* value	Case subjects	Control subjects	*P* value
Age at menarche, y	13.1 ± 1.6	13.2 ± 1.4	.636	13.0	13.3	.774
Age at first childbirth, y†	26.2 ± 3.4	26.6 ± 3.9	.676	26.0	26.8	.367
No. of births	1.8 ± 1.0	1.9 ± 1.1	.427	1.8	1.8	.559
Age at menopause, y	49.0 ± 6.4	48.4 ± 4.6	.669	48.2	49.2	.386
Weight, kg	68.1 ± 11.3	65.1 ± 10.9	.331	67.9	65.3	.291
Height, cm	156.4 ± 4.6	156.9 ± 6.0	.523	156.2	157.1	.914
Body mass index‡	27.9 ± 4.7	26.4 ± 4.5	.262	27.8	26.4	.294
Waist-to-hip ratio	0.83 ± 0.09	0.81 ± 0.05	.426	0.83	0.82	.503

*23 case subjects for the anthropometric measurements.
†Based on 20 parous case subjects.
‡Weight in kg/squared height in m.

and body mass index than control subjects; however, possibly because of lack of statistical power, none of the considered risk factors differed significantly between case and control subjects.

Table 2 shows the crude and age-adjusted mean hormone and SHBG levels in breast cancer case subjects and control subjects. The case subjects had significantly higher levels of total testosterone and free testosterone than the control subjects. After adjustment for age, the case subjects also had significantly higher levels of total E_2; moreover, they had higher levels of DHEAS and lower levels of SHBG, but the differences for these two variables were not statistically significant. As androgen (especially DHEAS) levels decreased with age, age-adjusted differences were larger than crude differences.

Table 3 shows RRs for breast cancer according to hormone serum level. For each hormone, the risk in the top and middle third is compared with that in the third with the lowest hormone levels. Table 3 also shows *P* values for test of trend. Higher levels of total testosterone and free testosterone were associated with statistically significant increases in the risk of breast cancer. After adjustment was made for age, women in the highest third of the distribution of total testosterone had an RR of 7.0 (95% confidence interval [CI] = 1.4-36.4) compared with women in the lower third. For women in the highest third of the distribution of free testosterone, the RR was 5.7 (95% CI = 1.5-22.2). An increase in the RR with increasing concentration of DHEAS and a decrease in the RR with increasing SHBG levels were also observed, but these trends were not statistically significant. The relationship between breast cancer risk and total E_2 serum levels was not linear, in that the RR was higher in the middle third of the distribution (7.1; 95% CI = 1.4-36.0).

We fitted several models that included one hormone, age, and one of the risk factors shown in Table 1 (one at a time). We found that the RRs associated with hormone levels did not differ greatly from those produced by the model that did not include the additional risk factor. Adjustment for years since menopause also had no effect on the RRs associated with hormone levels (data not shown). However, after adjustment for both age and body mass index, the RR tended to increase for total testosterone and to decrease for free testosterone. The risk for women with serum levels in the higher third of the distribution, relative to the risk for those with serum levels in the lower third, became 11.5 (95% CI = 1.3-99.6) for total testosterone and 4.6 (95% CI = 1.1-20.0) for free testosterone.

Serum levels of hormones were sometimes highly correlated: For control subjects, the r correlation coefficient of free testosterone with total testosterone was .58; with DHEAS, *r* equaled .49, whereas with total E_2, *r* equaled .12. We therefore attempted to fit several conditional logistic models that included two hormones and age. We found that RR associated with free testosterone decreased slightly when adjusted for total testosterone (for the highest third, RR from 5.7 [95% CI = 1.5-22.2] to 5.1 [95% CI = 0.8-33.4]). We found, however, that RRs for total testosterone, total E_2, and DHEAS decreased markedly when adjusted for free testosterone (for the highest third, RR from 7.0 [95% CI = 1.4-36.4] to 2.2 [95% CI = 0.3-17.3] for total testosterone, from 5.5 [95% CI = 0.8-37.6] to 2.1 [95% CI =0.2-25.2] for total E_2, and from 2.6 [95% CI = 0.6-11.1] to 0.9 [95% CI =

Table 2. Mean serum values of sex hormones in 24 postmenopausal case subjects and 88* matched control subjects from the ORDET cohort

	Crude mean ± standard deviation			Age-adjusted mean		
	Case subjects	Control subjects	*P* value	Case subjects	Control subjects	*P* value
Total testosterone, ng/mL	0.34 ± 0.22	0.25 ± 0.15	.011	0.34	0.25	<.001
Free testosterone, pg/mL	1.05 ± 0.46	0.79 ± 0.41	.007	1.07	0.77	.006
Total estradiol, pg/mL	24.0 ± 7.1	23.0 ± 10.2	.133	25.1	22.2	.027
Dehydroepiandrosterone sulfate, μg/mL	0.71 ± 0.49	0.73 ± 0.49	.806	0.79	0.66	.551
Sex hormone-binding globulin, nmol/L	44.2 ± 16.6	48.1 ± 20.2	.459	43.2	49.2	.423

*Because of insufficient serum, the analysis of total testosterone and free testosterone was based on 85 and 87 control subjects, respectively.

Table 3. Relative risks (RRs)* of breast cancer by level of serum sex hormones, based on 24 postmenopausal case subjects and 88† matched control subjects from the ORDET cohort

	Serum concentration			
	Low‡	Middle	High	*P* for trend
Total testosterone, ng/mL	<0.17	0.17-0.25	>0.25	
No. of case subjects	2	8	14	
Crude RR	1.0	3.7 (0.7-18.6)	6.3 (1.3-29.6)	.011
RR adjusted for age	1.0	4.8 (0.9-25.1)	7.0 (1.4-36.4)	.026
Free testosterone, pg/mL	<0.57	0.57-0.86	>0.86	
No. of case subjects	3	4	17	
Crude RR	1.0	1.3 (0.3-5.9)	5.9 (1.6-21.9)	.006
RR adjusted for age	1.0	1.8 (0.4-9.3)	5.7 (1.5-22.2)	.005
Total estradiol, pg/mL	<18.2	18.2-24.4	>24.4	
No. of case subjects	3	15	6	
Crude RR	1.0	5.7 (1.3-24.3)	2.4 (0.4-13.1)	.311
RR adjusted for age	1.0	7.1 (1.4-36.0)	5.5 (0.8-37.6)	.128
Dehydroepiandrosterone sulfate, μg/mL	<0.43	0.43-0.85	>0.85	
No. of case subjects	8	8	8	
Crude RR	1.0	1.0 (0.3-3.0)	1.0 (0.3-3.0)	.826
RR adjusted for age	1.0	2.0 (0.5-7.4)	2.6 (0.6-11.1)	.247
Sex hormone-binding globulin, nmol/L	<38.5	38.5-55.1	>55.1	
No. of case subjects	11	8	5	
Crude RR	1.0	0.7 (0.3-1.9)	0.5 (0.1-1.5)	.420
RR adjusted for age	1.0	0.6 (0.2-1.9)	0.3 (0.1-1.3)	.235

*The 95% confidence intervals of relative risk are given in parentheses.
†Because of insufficient serum, analysis of total testosterone and free testosterone was based on 85 and 87 control subjects, respectively.
‡Reference category.

0.2-5.0] for DHEAS). On the other hand, adjustment for total E_2, increased the association between breast cancer risk and levels of free testosterone and total testosterone (for the highest third, RR from 5.7 [95% CI = 1.5-22.2] to 5.9 [95% CI = 1.2-29.3] for free testosterone and from 7.0 [95% CI = 1.4-36.4] to 8.8 [95% CI = 1.2-63.8] for total testosterone).

Discussion

The results of the first 3.5 years of follow-up of the ORDET cohort provide direct evidence that high serum levels of both testosterone and E_2 precede breast cancer in postmenopausal women. They corroborate the hypothesis that steroid sex hormones are involved in breast carcinogenesis.

The study set out to investigate prospectively hormone levels in breast cancer. It was designed to eliminate as many sources of undesired variability as possible, by either restricting eligibility criteria, standardizing procedures, or matching.

We controlled for circadian variation in hormone levels by restricting collection time to 8:00 AM to 9:30 AM. We have shown, however, that both E_2 and testosterone levels decrease considerably between 8:00 AM and 9:00 AM (*15*); therefore, we recorded the exact time of venipuncture. On average, the time of venipuncture was slightly later for case subjects than for control subjects. We therefore included this variable in a logistic model. The adjustment increased RR estimates for high total testosterone and free testosterone and, to a lesser extent, for total E_2 (data not shown).

Matching case and control subjects proved to be important for controlling undesired variability. Mean free testosterone was significantly higher ($P<.001$) and mean SHBG was significantly lower ($P = .04$) in the samples transported between study centers for processing than in the samples collected and processed at the same study center. Restricting the analysis to subjects whose blood had not been transported (16 case subjects and 59 control subjects), we found that the RRs for breast cancer still significantly increased with free testosterone and total testosterone concentrations in spite of a loss of statistical power: For the highest third, the age-adjusted RRs were 5.3 (95% CI = 1.4-20.7) for free testosterone and 6.9 (95% CI = 1.2-40.6) for total testosterone. Mean SHBG levels were higher in samples collected in standard time than in those collected in daylight saving time (summertime) ($P = .017$). This finding may be related to the fact that the diet of northern Italians is different in fall–winter than in spring–summer. A vegetarian diet is associated with high SHBG levels (*16*). The mean concentration of free testosterone in samples stored at the top of freezers was higher than that in samples stored at the bottom, but the difference was not statistically significant.

Given the prospective design of the study, any effect of clinical breast cancer on hormone levels, either directly or through behavioral changes, can be ruled out. Although all participants underwent physical breast examination at enrollment and were excluded if breast cancer was detected, some women with early breast cancer may have been included. To examine whether the strong associations reported in Table 3 could be explained by latent preclinical cases, we restricted the analysis to the 14 case subjects diagnosed 6 or more months after enrollment and their matched control subjects. We found that age-adjusted RR for free testosterone levels increased to 3.7 (95% CI = 0.3-40.2) in the middle third of the distribu-

tion and to 8.4 (95% CI = 1.0-72.1) in the upper third.

We are aware of four other published prospective studies (*5-8*) on serum sex hormone levels and postmenopausal breast cancer. The first to be published (*5*) was based on 12 case subjects and suggested a strong positive association between breast cancer and free E_2 levels and a negative association between breast cancer and SHBG levels. Recently, such associations were confirmed in a much larger study (*8*) of 130 breast cancer cases occurring in the first 5.5 years of follow-up of the Women's Health Cohort at New York University: RRs of breast cancer for increasing quartiles of free E_2 were 1.0, 1.5, 3.8, and 3.9 (P for trend <.001). The association with total E_2, however, was less strong. These results are consistent with our findings on free testosterone, since testosterone has a much stronger affinity for SHBG than E_2 (*17*); moreover, testosterone decreases hepatic synthesis of SHBG. High free E_2 levels are therefore expected in the presence of high free testosterone; Takatani et al. (*18*) found that the correlation coefficient between these two variables was r = .82. Two further studies (*6,7*) were not specifically designed to investigate hormones and breast cancer but analyzed sera stored for other purposes. One of these studies (*6*), which was based on 15 incident cases, showed no breast cancer association with levels of estrogens or androgens. The other (*7*), which was based on 29 case subjects, showed a statistically significant positive association between breast cancer and dehydroepiandrosterone serum levels (*19*) but not estrogen levels (*7*); nevertheless, the analysis showed that the RR for breast cancer increased up to four times with increasing blood levels of total and free E_2, a result consistent with ours. In both studies (*6,7*), the time of follow-up (up to 15 and 17 years, respectively) was much longer than in our investigation, so that hormone levels were determined from a single blood sample taken many years earlier. Furthermore, in the first study (*6*), serum samples were preserved at the top of freezers that were used for other purposes, and in the second (*7*), the interval between blood drawing and processing could be as long as 24 hours, which may have affected sex hormone levels. None of these four studies (*5-8*) controlled for circadian variation.

Hormone analyses were based on a single venipuncture, which may not guarantee a reliable assessment of average longterm levels. Our present and previous (*20*) repeatability studies and those of others (*21,22*) show that in postmenopausal women, the intraclass correlation between values from two measurements taken from one subject 1 or 2 years apart is good for total testosterone, DHEAS, and free steroid sex hormones (range, .7-.9) but only fair for total E_2 (range, .5-.7). The association between breast cancer and high total E_2 levels observed in epidemiologic studies, therefore, may be somewhat underestimated.

The mechanism by which estrogens can promote breast cancer growth is clearly recognized, but the role of androgens is less clear. Androgens may be important as estrogen precursors, but there is also evidence that androgens can directly stimulate the growth of human breast cancer cell lines (*23*). The climacteric ovary has stopped producing estrogens but continues to secrete androgen. It has been estimated that the ovaries are responsible for 40% of total testosterone after menopause; the remainder is produced by the adrenals (*24*). That this ovarian activity is relevant to breast cancer etiology is supported by the observation that external-beam irradiation of the climacteric ovary is associated with lower levels of circulating testosterone (*25*) and with a lower risk of breast cancer (*26*).

In conclusion, our study has produced two important findings. First, in our population, high levels of steroid sex hormones were found to be powerful predictors of breast cancer. Second, although the association between breast cancer risk and testosterone levels was already known from case–control studies (*18,27*), our results prospectively confirm this association. With only 24 cases, however, uncertainty remains about the magnitude of the association, especially when several possible confounders are considered simultaneously. Follow-up of our cohort continues. Further analysis is planned in 1996, when we will assay free E_2, which was not measured in the present study because of the limitations imposed by the amounts of sera necessary for this study. Meanwhile, the whole cohort is being recruited for a second blood sample and dietary questionnaire. The possibility that both androgens and bioavailable estrogens are involved in breast carcinogenesis is appealing and may open the way to new approaches to preventing breast cancer. However, further studies of the predictive value of steroid sex hormone levels are vital, as are studies of the genetic and environmental determinants of hormone levels.

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Notes

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