[CANCER RESEARCH 64, 6814–6819, September 15, 2004]

Plasma Prolactin Concentrations and Risk of Postmenopausal Breast Cancer

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ABSTRACT

Prolactin is important in human breast development, and substantial laboratory and *in vitro* data suggest a role in mammary carcinogenesis. Therefore, we conducted a prospective case-control study nested within the Nurses' Health Study cohort to examine, in detail, the association between plasma prolactin concentrations and postmenopausal breast cancer by cancer invasiveness, estrogen receptor/progesterone receptor status, and other subject characteristics, including postmenopausal hormone use. Blood samples were collected from 1989 to 1990 and prolactin was measured by microparticle enzyme immunoassay. The analysis included 851 cases of postmenopausal breast cancer diagnosed after blood collection and before June 2000, in which there were one or two controls (n = 1,275) matched on age, postmenopausal hormone use, fasting status, and time of day and month of blood collection. Prolactin was associated with a modestly increased risk of postmenopausal breast cancer [relative risk, top *versus* bottom quartile, 1.34; 95% confidence interval (CI), 1.02–1.76; P-trend = 0.01]. The association differed by estrogen receptor/progesterone receptor status (P-heterogeneity = 0.03). The relative risk was 1.78 (95% CI, 1.28, 2.50; P-trend < 0.001) for estrogen receptor+/progesterone receptor+, 0.76 (95% CI, 0.43, 1.32; P-trend = 0.28) for estrogen receptor−/progesterone receptor−, and 1.94 (95% CI, 0.99, 3.78; P-trend = 0.12) for estrogen receptor+/progesterone receptor− breast cancers. Associations generally were similar for ductal and lobular carcinomas (P-heterogeneity = 0.43) and by tumor size (P-heterogeneity = 0.24). Among estrogen receptor+/progesterone receptor+ cancers, the association did not significantly differ by postmenopausal hormone use, years between blood draw and diagnosis, or after adjustment for estradiol (relative risk, 1.93; 95% CI, 1.16, 3.22; P-trend = 0.01). Our prospective data suggest that plasma prolactin concentrations are associated with an increased risk of postmenopausal breast cancer, particularly for estrogen receptor+/progesterone receptor+ cancers, and independently of estradiol.

INTRODUCTION

Prolactin and other sex hormones, such as estradiol and progesterone, are important in normal mammary gland growth and development, as well as lactation (1). Both animal and *in vitro* data suggest that prolactin is involved in tumorigenesis (2) by promoting cell proliferation (3–5), increasing cell motility (6), and improving tumor vascularization (2, 7). Whereas prolactin and its receptor are found in normal and malignant tissues, concentrations of both are generally higher in malignant tissue (5, 8–10).

Epidemiologic data are somewhat limited. In the initial report from the Nurses' Health Study, we reported that postmenopausal women in the highest quartile of prolactin concentrations had an increased risk of breast cancer compared with those in the lowest quartile (relative risk = 2.03; P-trend = 0.01) among 306 breast cancer cases over 4 years of follow-up (11). Two small prospective studies (n = 26 and 40 cases) reported a nonsignificantly increased breast cancer risk with higher prolactin concentrations (12, 13). Manjer *et al.* (14) reported no consistent association across quartiles of prolactin, with an odds ratio in the top *versus* bottom quartile of 1.34 [95% confidence interval (CI), 0.83–2.17] among 173 cases. Results of case-control studies have been conflicting, likely because of small sample sizes and the probable influence of breast cancer on prolactin concentrations (2).

Previous studies have not been large enough to consider whether the association may differ among various subgroups of breast cancer or by other subject characteristics. Therefore, we conducted a prospective case-control study nested within the Nurses' Health Study cohort to examine, in further detail, the association between plasma prolactin concentrations and postmenopausal breast cancer by cancer invasiveness, estrogen/progesterone receptor status, and other subject characteristics, including postmenopausal hormone use and antidepressant use. This study includes an additional 445 postmenopausal breast cancer cases compared with our previous report (11).

MATERIALS AND METHODS

Study Population. The Nurses' Health Study cohort was established in 1976 when 121,700 United States female registered nurses, ages 30 to 55 years, completed and returned a mailed questionnaire. The Nurses' Health Study cohort has been followed every 2 years since inception by questionnaire to update exposure variables and ascertain newly diagnosed disease. Data have been collected on various breast cancer risk factors such as weight, height, age at menarche, parity, age at first birth, age at menopause, postmenopausal hormone use, and family history of breast cancer.

Between 1989 and 1990, 32,826 cohort members provided blood samples; women were between 43 and 69 years of age at blood collection. Details about the blood collection methods have been published previously (15). Briefly, women arranged to have their blood drawn and shipped with an ice pack, via overnight courier, to our laboratory where it was processed and separated into plasma, red blood cell, and white blood cell components. Seventy percent of samples were collected while fasting for >8 hours, and 97% were received within 26 hours of collection. The stability of prolactin in whole blood for 24 to 48 hours has been shown previously (16). Samples have been stored in continuously monitored, liquid nitrogen freezers since collection. At blood collection, women completed a short questionnaire asking about current weight, postmenopausal hormone use, and the use of antidepressant medication. Follow-up of the blood study cohort was 99% in 2000.

Both cases and controls were postmenopausal at the time of blood collection. Women were considered to be postmenopausal if they: (1) reported having a natural menopause (*e.g.*, no menstrual cycles during the previous 12 months), (2) had a bilateral oophorectomy, or (3) had a hysterectomy but had at least one ovary remaining, and were at least 56 (for nonsmokers) or 54 (for smokers) years of age (11). These were the ages at which natural menopause occurred for 90% of the overall cohort.

Cases had no reported cancer diagnosis before blood collection and were diagnosed with breast cancer after blood collection but before June 1, 2000. In all, 861 cases of postmenopausal breast cancer, with known postmenopausal hormone status at blood draw, were reported and confirmed by medical record review (n = 847) or by verbal confirmation of the diagnosis by the nurse (n = 14). Due to the high confirmation rate in medical review (99%), these latter cases were included in the analysis. Time from blood draw to diagnosis ranged from 1 month to 151 months (mean, 67.0 months). Cases and controls were matched on age (±2 years), recent postmenopausal hormone use, month/year of blood collection (±1 month), time of day of blood draw (±2 hours), and fasting status (≥10 hours since last meal, <10 hours since last meal, and

Received 5/27/04; revised 7/2/04; accepted 7/15/04.

Grant support: NIH Grants P01 CA87969, CA49449, P50 CA089393, and DAMD-17-02-1-0692. S. Tworoger was partially supported by Training Grant in Cancer Epidemiology T32 CA090001 from the National Cancer Institute.

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unknown). For cases ($n = 447$) who reported using postmenopausal hormones <3 months before blood collection (*i.e.,* "recent postmenopausal hormone use"), one control was matched per case, and for cases ($n = 414$) who did not report recent postmenopausal hormone use at blood collection, two controls were matched per case; this was done to increase power in analyses only using the latter case group. Exact control subject matches were obtained for 97% of cases on age, 94% on time of day, and 95% on month of blood collection. The most relaxed matches were ±6 years of age, ±12 hours, and ±14 months, respectively. Forty-five control women went on to subsequently develop breast cancer; however, we have only included these individuals as controls.

Reproducibility Study. Among women providing a blood sample in 1989 to 1990, 390 participants were asked to collect two additional samples over the following 2 to 3 years. Participants were postmenopausal, had no prior diagnosis of cancer (except nonmelanoma skin cancer), and had no history of recent postmenopausal hormone use at each sample collection. Of the 390 invited subjects, 186 (48%) sent two additional samples. A random sample of 80 of these women who had all three of the samples drawn between 6 a.m. and 12 p.m. was sent for prolactin analysis and forms the basis of the reproducibility study. Details regarding this study have been published elsewhere (17).

Laboratory Assays. Prolactin was measured using a microparticle enzyme immunoassay. The laboratory of Dr. Christopher Longcope at the University of Massachusetts Medical Center (Boston, MA) assayed 164 cases and 245 controls, in three batches, using the IMx System (Abbott Laboratory, Abbott Park, IL), between March 1993 and August 1997. The remaining samples (697 cases and 1,051 controls) were assayed, in three batches, at the Reproductive Endocrinology Unit Laboratory at the Massachusetts General Hospital, using the AxSYM Immunoassay system (Abbott Diagnostics, Chicago, IL), between August 2001 and November 2003. A subset of 60 samples was assayed at each laboratory; the correlation between the two laboratories was 0.91. The limit of detection (for both laboratories) was 0.6 ng/mL; 1 sample had a value below this limit. Estrone and estradiol were assayed by sensitive and specific radioimmunoassays following organic solvent extraction and Celite column partition chromatography among cases and controls who were not using postmenopausal hormones at blood draw; these methods are described in detail elsewhere (18).

All of the case-control pairs (or triplets) were assayed together, with a random sample order. Laboratory technicians were blinded to case-control status. In each batch we included replicate plasma samples to assess laboratory precision. The intra-assay coefficient of variation ranged from 5.4% to 9.3%. In the final (sixth) batch assayed in 2003 we included 15 control plasma samples from each of the previous five batches, hereafter referred to as drift samples, to assess laboratory drift.

Statistical Analysis. Mean plasma prolactin concentrations from the drift samples differed by batch, indicating that there was some laboratory drift over time. Therefore, using the drift samples, we recalibrated prolactin values from the first five batches to have a comparable distribution to the final batch. To do this we used linear regression, separately by batch, to assess the relationship between the assay value measured in the final batch to that measured in the original batch and used the intercept and β coefficient to rescale all of the values in the original batch. We then created quartile cut points based on all of the controls using the recalibrated prolactin values. Results using these data *versus* using batch-specific quartile cut points from the original data were very similar; therefore, unless otherwise specified, we present the results using the recalibrated data.

We excluded women who were missing prolactin values related to technical difficulties with the assay ($n = 9$ cases and 16 controls). We identified statistical outliers based on the generalized extreme studentized deviate many-outlier detection approach (19); women with prolactin concentrations >74 ng/mL ($n = 1$ case and 4 controls) or <0.6 ng/mL ($n = 1$ control) were excluded. Overall, 851 cases and 1,275 controls were available for analysis.

For our primary analysis, we used conditional logistic regression to estimate odds ratios and 95% CI comparing quartiles of prolactin concentrations (20). The odds ratios appropriately estimate the relative risks because the outcome is rare; therefore, we henceforth use the term relative risk. In addition, we estimated relative risks and 95% CIs comparing quartiles of prolactin concentrations across various case groups (*in situ versus* invasive, ductal *versus* lobular, tumor size ≤2 cm *versus* > 2 cm, estrogen receptor/progesterone receptor status, and time between blood draw and diagnosis) using polytomous unconditional logistic regression adjusting for matching factors (21). To determine whether the relative risks across case groups differed, we compared a model holding the association of log-transformed prolactin and breast cancer constant across case groups to one allowing the association to vary, using the likelihood ratio test (21). For time between blood draw and diagnosis, we also conducted a trend test comparing the slopes for log-transformed prolactin concentrations across case groups (22, 23). Secondary, *a priori*, analyses, excluding women with a high prolactin level (>24 ng/mL) or taking antidepressants and stratifying by postmenopausal hormone use used unconditional logistic regression adjusting for matching factors. Using continuous, log-transformed original data (*e.g.,* nonrecalibrated data), we corrected the point and interval estimates for laboratory measurement error and random within-person variation (24). We calculated the within-person variance using the reproducibility study data and the between-person variance using the case-control study data to obtain an intraclass correlation of 0.49.

All of the models were adjusted for the following *a priori* potential confounders: body mass index at age 18 (<21, 21-<23, 23-<25, ≥25 kg/m^2, or missing), weight change from age 18 to blood draw (<5, 5-<20, ≥20 kg, or missing), family history of breast cancer (yes or no), age at menarche (<12, 12, 13, or ≥14 years), age at first birth/parity (nulliparous, age at first birth <25 years/1–4 children, age at first birth 25–29 years/1–4 children, age at first birth ≥30 years/1–4 children, age at first birth <25/≥5 children, or age at first birth ≥25/≥5 children), and age at menopause (<45, 45–49, 50–54, or ≥55 years). Additional adjustment for oophorectomy, history of benign breast disease, duration of oral contraceptive use, or duration of postmenopausal hormone use did not substantially alter the results. Although we adjusted for parity, which may be part of the biological pathway through which prolactin affects breast cancer (2), it did not alter the risk estimates. Tests for trend were conducted by modeling log-transformed prolactin concentrations continuously and calculating the Wald statistic (25). All of the *P*s were based on two-sided tests and were considered statistically significant if ≤0.05.

RESULTS

Subjects were 45 to 70 years of age (mean, 61 years) at blood collection (Table 1). Differences between cases and controls for age at menarche, age at menopause, parity, and body mass index at age 18 or blood draw generally were small, although in the expected direction. A higher percentage of cases *versus* controls had a family history of breast cancer (26.0% *versus* 18.6%, respectively) and a history of benign breast disease (40.5% *versus* 33.8%, respectively). Cases also had a higher median prolactin concentration than controls ($P < 0.001$).

There was a modest positive association between plasma prolactin concentrations and breast cancer for all of the subjects (*P*-trend = 0.01; Table 2). The multivariate relative risk in the top *versus* bottom quartile was 1.34 (95% CI, 1.02, 1.76). This relative risk was slightly attenuated after excluding cases who were diagnosed within 2 years of their blood collection (relative risk, 1.28; 95% CI, 0.98, 1.67; *P*-trend = 0.02), whereas the relative risk was strengthened slightly (relative risk, 1.39; 95% CI, 1.07, 1.82; *P*-trend = 0.006) after

Table 1 *Characteristics at blood collection of cases and their matched control subjects from the Nurses' Health Study*

	Case women ($n = 851$), mean (SD)	Control women ($n = 1,275$), mean (SD)
Age (y)	60.7 (5.1)	61.0 (4.9)
Age at menarche (y)	12.5 (1.4)	12.6 (1.4)
Age at menopause (y)	48.0 (5.6)	47.8 (5.8)
Parity*	3.3 (1.6)	3.4 (1.6)
BMI at age 18 (kg/m^2)	21.2 (2.8)	21.4 (2.9)
BMI at blood draw (kg/m^2)	25.7 (4.8)	25.6 (4.6)
Family history of breast cancer, %	26.0	18.6
History of benign breast disease, %	40.5	33.8
Took anti-depressant medication, %	4.2	4.9
Median prolactin, ng/mL (10^{th}–90^{th} percentile)	10.2 (6.2–18.5)	9.4 (6.1–16.7)

Abbreviation: BMI, body mass index.
* Among parous women only.

Table 2 *Relative risk (95% CIs) of breast cancer by quartile of plasma prolactin concentration among postmenopausal women in the Nurses' Health Study*

	n, case/control	Prolactin Concentrations				*P* for trend*
		≤7.4 ng/mL	>7.4–9.4 ng/mL	>9.4–12.3 ng/mL	>12.3 ng/mL	
Simple relative risk	851/1,275	1.0 (ref.)	0.88 (0.67, 1.16)	1.18 (0.91, 1.53)	1.34 (1.03, 1.74)	0.01
Multivariate relative risk†	851/1,275	1.0 (ref.)	0.90 (0.68, 1.19)	1.18 (0.90, 1.54)	1.34 (1.02, 1.76)	0.01
Excluding cases diagnosed within 2 years of blood draw‡	723/1,275	1.0 (ref.)	0.76 (0.57, 1.01)	1.04 (0.79, 1.36)	1.28 (0.98, 1.67)	0.02
Excluding women with prolactin > 24 ng/mL‡	814/1,232	1.0 (ref.)	0.86 (0.66, 1.12)	1.20 (0.93, 1.56)	1.39 (1.07, 1.82)	0.006

* Determined using continuous, log-transformed prolactin concentrations.
† Adjusted for body mass index at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, age at first birth/parity, and age at menopause.
‡ Adjusted for body mass index at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, age at first birth/parity, age at menopause and matching factors.

excluding women with a prolactin concentration >24 ng/mL, which is the top end of the normal range.

Results were similar when removing women (n = 115 cases and 152 controls) who were taking or unsure if they were taking antidepressant medication, which can alter prolactin concentrations (2), at blood collection (data not shown); comparing the top *versus* bottom quartile, the relative risk was 1.36 (95% CI, 1.03, 1.80; *P*-trend = 0.01). Correcting for measurement error and within-person variability, the relative risk increased from 1.28 (95% CI, 1.05, 1.57) to 1.68 (95% CI, 1.10, 2.55) for a one-unit increase in log-transformed prolactin concentrations.

The relationship between plasma prolactin and breast cancer appeared to vary by *in situ versus* invasive cancers (*P*-heterogeneity = 0.11; Table 3). Overall there was no association for *in situ* cancers (*P*-trend = 0.84) but a significant positive association for invasive cancers (*P*-trend = 0.003), with a relative risk of 1.41 (95% CI, 1.08, 1.86) comparing the top to bottom quartiles. The relationship between plasma prolactin and invasive breast cancer was similar for ductal *versus* lobular types (*P*-heterogeneity = 0.43) and for tumors ≤2 cm compared with tumors >2 cm (*P*-heterogeneity = 0.24). However, the risk significantly differed by estrogen receptor and progesterone receptor status (*P*-heterogeneity = 0.03). The relative risk in the top *versus* bottom quartile of plasma prolactin was 1.78 (95% CI, 1.28, 2.50; *P*-trend < 0.001) for estrogen receptor+/progesterone receptor+ cases, 0.76 (95% CI, 0.43, 1.32; *P*-trend = 0.28) for estrogen receptor−/progesterone receptor− cases, and 1.94 (95% CI, 0.99, 3.78; *P*-trend = 0.12) for estrogen receptor+/progesterone receptor− cases. There were too few estrogen receptor−/progesterone receptor+ cases (n = 18) to consider separately. Among estrogen receptor+/progesterone receptor+ cases, correcting for laboratory error and within-person variability, the relative risk increased from 1.61 (95% CI, 1.26, 2.06) to 2.77 (95% CI, 1.62, 4.73) for a one-unit increase in log-transformed prolactin concentrations.

Among estrogen receptor+/progesterone receptor+ cases, we found that the association did not differ by postmenopausal hormone use at blood draw (*P*-interaction = 0.41 comparing past to never users and 0.77 comparing current to never users; Table 4). Although there was no statistical difference by time between diagnosis and blood collection (*P*-heterogeneity = 0.67), the association appeared to be stronger in the first few years after blood collection; the relative risk for the top *versus* bottom quartile was 3.10 for 0 to 2 years, 2.23 for 2 to 4 years, 1.56 for 4 to 8 years, and 1.58 for 8+ years (*P*-trend = 0.12). In a subset of women with measured estrogen concentrations, the relative risk was essentially unchanged compared with all of the women with estrogen receptor+/progesterone receptor+ tumors. Additional adjustment for estrone or estradiol concentrations also did not alter the results. Results were similar when considering all

Table 3 *Multivariate* relative risk (95% CI) of breast cancer by quartile of plasma prolactin concentration among postmenopausal women in the Nurses' Health Study by invasiveness, type, tumor size, and receptor status†*

	Prolactin Concentrations					
	≤7.4 ng/mL	>7.4–9.4 ng/mL	>9.4–12.3 ng/mL	>12.3 ng/mL	*P* for trend‡	*P* for heterogeneity§
In situ (n = 115 cases)	1.0 (ref.)	0.85 (0.49, 1.46)	0.80 (0.46, 1.39)	0.96 (0.57, 1.63)	0.84	0.11
Invasive (n = 722 cases)	1.0 (ref.)	0.84 (0.63, 1.12)	1.27 (0.97, 1.67)	1.41 (1.08, 1.86)	0.003	
Ductal (n = 588 cases)	1.0 (ref.)	0.85 (0.62, 1.15)	1.19 (0.89, 1.60)	1.38 (1.04, 1.85)	0.007	0.43
Lobular (n = 93 cases)	1.0 (ref.)	0.86 (0.42, 1.76)	1.69 (0.90, 3.14)	1.76 (0.95, 3.26)	0.11	
Tumor size ≤2 cm (n = 531 cases)	1.0 (ref.)	0.87 (0.63, 1.19)	1.22 (0.91, 1.65)	1.35 (1.00, 1.83)	0.01	0.24
Tumor size >2 cm (n = 162 cases)	1.0 (ref.)	0.74 (0.43, 1.28)	1.35 (0.84, 2.19)	1.66 (1.04, 2.64)	0.03	
ER+/PR+ (n = 397 cases)	1.0 (ref.)	0.87 (0.60, 1.26)	1.52 (1.08, 2.13)	1.78 (1.28, 2.50)	<0.001	0.03
ER−/PR− (n = 96 cases)	1.0 (ref.)	0.49 (0.26, 0.92)	0.73 (0.41, 1.28)	0.76 (0.43, 1.32)	0.28	
ER+/PR− (n = 91 cases)	1.0 (ref.)	1.76 (0.90, 3.47)	1.74 (0.88, 3.43)	1.94 (0.99, 3.78)	0.12	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; BMI, body mass index.
* Adjusted for BMI at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, age at first birth/parity, age at menopause, and matching factors.
† Too few ER−/PR+ cases (n = 18) were available to analyze separately.
‡ Determined using continuous, log-transformed prolactin concentrations.
§ Determined using polytomous logistic regression and the likelihood ratio test, comparing a model constraining relative risks to be the same across all case groups *versus* a model allowing the relative risks to differ across case groups.

Table 4 *Multivariate* relative risks (95% CI) for ER+/PR+ breast cancers by quartile of plasma prolactin concentration among postmenopausal women in the Nurses' Health Study*

	n, case/control	Prolactin Concentrations				*P* for trend†
		≤7.4 ng/mL	>7.4–9.4 ng/mL	>9.4–12.3 ng/mL	>12.3 ng/mL	
By postmenopausal hormone use at blood draw‡						
Never user	111/545	1.0 (ref.)	1.04 (0.54, 1.97)	1.74 (0.95, 3.16)	1.89 (1.02, 3.50)	0.16
Past user	67/277	1.0 (ref.)	1.00 (0.42, 2.40)	1.76 (0.82, 3.78)	2.33 (1.06, 5.14)	0.02
Current user	219/453	1.0 (ref.)	0.76 (0.44, 1.31)	1.29 (0.79, 2.12)	1.55 (0.98, 2.47)	0.01
By time between blood draw and diagnosis (for cases)§						
0–2 y	52/1,275	1.0 (ref.)	2.01 (0.68, 5.98)	4.23 (1.57, 11.4)	3.10 (1.11, 8.62)	0.04
2–4 y	74/1,275	1.0 (ref.)	0.77 (0.33, 1.80)	1.63 (0.80, 3.33)	2.23 (1.13, 4.41)	0.02
4–8 y	161/1,275	1.0 (ref.)	0.67 (0.39, 1.16)	1.20 (0.75, 1.94)	1.56 (0.99, 2.47)	0.02
8+ y	110/1,275	1.0 (ref.)	1.05 (0.57, 1.94)	1.33 (0.74, 2.38)	1.58 (0.90, 2.79)	0.03
Adjusting for estrone or estradiol among never and past postmenopausal hormone users						
Not adjusted	175/784	1.0 (ref.)	1.00 (0.59, 1.69)	1.73 (1.06, 2.81)	1.99 (1.20, 3.30)	0.01
Estrone	131/597	1.0 (ref.)	0.82 (0.44, 1.54)	1.64 (0.92, 2.92)	2.18 (1.21, 3.94)	0.02
Estradiol	175/784	1.0 (ref.)	0.98 (0.57, 1.66)	1.77 (1.08, 2.90)	1.93 (1.16, 3.22)	0.01

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; BMI, body mass index.

* Adjusted for BMI at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, age at first birth/parity, age at menopause, and matching factors.

† Determined using continuous, log-transformed prolactin concentrations.

‡ The *P*-interaction between past and never users was 0.41 and between current and never users was 0.77.

§ The *P*-heterogeneity was 0.67, determined using polytomous logistic regression and the likelihood ratio test, comparing a model constraining relative risks to be the same across all case groups *versus* a model allowing the relative risks to differ across case groups.

of the estrogen receptor+ cases together, regardless of progesterone receptor status (data not shown).

DISCUSSION

This is the largest prospective study to date examining the association between plasma prolactin concentrations and postmenopausal breast cancer and the first to evaluate relationships by tumor characteristics. We observed a positive association between prolactin and breast cancer risk overall. However, the increased risk appeared to be confined to invasive cancers, particularly those tumors that were estrogen receptor+/progesterone receptor+ and estrogen receptor+/progesterone receptor−. Also, the association appeared strongest for estrogen receptor+/progesterone receptor+ cases diagnosed within 2 years of blood collection, although we still observed a positive association among tumors diagnosed 8 or more years after blood collection.

The overall positive association that we observed between plasma prolactin and breast cancer generally is consistent with previous studies. In an earlier analysis of this same dataset, consisting of 306 cases, the relative risk comparing the top to bottom quartile was stronger than in the present study (relative risk, 2.03; 95% CI, 1.24, 3.31); the risk was slightly stronger after excluding *in situ* cancers (11), which is similar to the results of the present study. Wang *et al.* (13) reported a nonsignificant increased breast cancer risk comparing the top to the bottom quintile of prolactin, with a relative risk of 1.63 (95% CI, 0.57, 4.71); Kabuto *et al.* (12) also reported a nonsignificant increased risk. The lack of statistical significance in both studies is probably due to the small number of cases available for analysis ($n = 40$ and 26 cases, respectively). One prospective nested case-control study with 173 *in situ* and invasive cases and 438 controls found no consistent association after adjustment for matching factors and body mass index (14). However, the odds ratio in the top *versus* bottom quartile (1.34; 95% CI, 0.83, 2.17) was similar to the relative risk that we observed when including all of the cases.

Both animal and *in vitro* models support the hypothesis that prolactin is involved in mammary carcinogenesis. Several studies have reported that breast cancer cells/tissue express prolactin (9, 26–28) and the prolactin receptor (8–10, 28). Although normal tissue also expresses the prolactin receptor, primarily along the luminal cell border, several studies have reported higher levels in tumor tissue (10, 29) with expression primarily in the cytoplasm (8). In mice, prolactin appears to induce tumor formation (30, 31), increase tumor growth rate (5), and increase the number of cells in the S phase (31). *In vitro* studies also suggest that prolactin is associated with higher cell proliferation rates (3–5), increases in cyclin D1 (3, 4), and it may induce motility of breast cancer cell lines (6). Preliminary data also suggest that prolactin can enhance the responsiveness of breast cancer cells to estradiol (3). In humans, prolactin concentrations were positively associated with mammographic density, a consistent strong breast cancer risk factor (32), among 189 postmenopausal women after adjustment for age and waist circumference (33). Paradoxically, prolactin is temporarily increased during breastfeeding, which is a protective factor for breast cancer. One possible explanation for this discrepancy is that pregnancy is associated with a lifetime decrease in prolactin levels (2), and this may outweigh the transient prolactin increase during breastfeeding. Secondly, the effect of prolactin during breastfeeding may differ from its effect at other times in the reproductive life of a woman; for example, it may lead to terminal cell differentiation during lactation but not at other times.

We found that the increased risk of postmenopausal breast cancer associated with high prolactin concentrations was confined primarily to invasive cancers, particularly estrogen receptor+/progesterone receptor+ and estrogen receptor+/progesterone receptor− tumors. Of the multiple functions of prolactin, it is possible that increasing survival and motility are the predominate effects on tumor cells, which would promote increased invasion into the surrounding stromal tissue (2). Several (8, 10, 34–37), but not all (9, 38–40), studies have reported that the prolactin receptor and estrogen receptor are coex-

pressed; results for the coexpression with progesterone receptor are less clear (8, 10, 36, 37, 39, 40). Differences between studies may be due, at least in part, to the different methods of detecting the presence of the prolactin receptor (2, 10). The mechanism underlying coexpression of the prolactin receptor and estrogen receptor is unclear. Several *in vitro* studies have reported that long-term prolactin exposure can increase estrogen receptor expression (3, 10). Rose-Hellekant *et al.* (31) reported that transgenic mice with constant prolactin expression developed both estrogen receptor+ and estrogen receptor− tumors, although estrogen receptor+ tumors are extremely rare in this mouse model. These data taken together suggest that prolactin may be important in the development of estrogen receptor+ tumors.

We found that among estrogen receptor+/progesterone receptor+ tumors, the association of prolactin did not differ by postmenopausal hormone use, although postmenopausal hormone can increase prolactin concentrations (11). This is particularly interesting given that the association between estrogens and postmenopausal breast cancer risk appears to be stronger in never-postmenopausal hormone users compared with past users (18, 41). We also found that prolactin concentrations predict breast cancer risk independently of estrone or estradiol concentrations among never and past users of postmenopausal hormones at blood collection.

Whereas we observed an association between prolactin and estrogen receptor+/ progesterone receptor+ breast cancers diagnosed >8 years after blood collection, the association appeared to be strongest among cases diagnosed within 2 years of blood collection. If recent/current levels are most important for risk then the risk estimates will be attenuated in later years, because the within-woman reproducibility decreases over time, introducing increased measurement error. Alternatively, our results are consistent with the possibility that subclinical breast cancer may increase prolactin concentrations. Bhatavdekar *et al.* (28) reported that immunohistochemical staining of prolactin in breast tumors was correlated with plasma prolactin concentrations ($r = 0.41$). Thus, it is important for studies examining the relationship between prolactin and breast cancer to use a prospective design, especially in light of other evidence that prolactin secretion can be influenced by physical or psychological stress after diagnosis (42–44).

This study has several limitations. First, several forms of prolactin circulate in human plasma, which appear to have different biological activities (45, 46). The immunoassay used in this study, which identify most prolactin isoforms (47), cannot distinguish between them. Therefore, we cannot isolate which isoform or isoforms are most important in breast cancer development. Second, although we used a high-precision assay, there was laboratory drift over time, potentially precluding us from considering the relationship of absolute prolactin concentrations with breast cancer. However, the addition of drift samples in the final batch of assays allowed us to recalibrate previous batches so that we could consider the relative concentrations. Third, prolactin has a strong circadian rhythm (48) and increases after a noontime meal (49). To minimize misclassification related to time of day of blood draw and fasting status, we closely matched case and control subjects on both of these factors. Also, prolactin values tend to fluctuate over time within a woman (intraclass-correlation over 3 years = 0.49) among postmenopausal women (17), which could attenuate the risk estimates. Therefore, we used information from a reproducibility study to correct our relative risk estimates for the random, biological variation in prolactin concentrations that cannot be captured by a single hormone measurement.

This is the largest prospective epidemiologic study of plasma prolactin concentrations and breast cancer, which allowed us to consider whether the risk differed by various subtypes of breast cancer tumors or other subject characteristics. We demonstrated a moderate positive association between plasma prolactin concentrations and the risk of invasive postmenopausal breast cancer, which is independent of estrogen concentrations. Furthermore, it appears that prolactin is associated primarily with estrogen receptor+ tumors. Our study lends substantial support to the hypothesis that prolactin is important in breast cancer etiology, although additional confirmation in other prospective studies is needed. It is also important to study whether prolactin may be important in the development of premenopausal breast cancer.

ACKNOWLEDGMENTS

We thank Victor Pontes, Helena Judge Ellis, and Gloria Sin for their expert assistance and Drs. Graham Colditz and Walter Willett for their helpful comments on this manuscript. We also thank the participants of the Nurses' Health Study for their longstanding contributions.

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Cancer Res 2004;64:6814-6819.