Association between Plasma Prolactin Concentrations and Risk of Breast Cancer among Predominately Premenopausal Women

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Abstract

Recent evidence suggests that prolactin may be positively associated with postmenopausal breast cancer risk; however, little data are available in younger women. Therefore, we conducted a prospective, nested case-control study to examine the relationship between plasma prolactin concentrations and breast cancer risk in predominately premenopausal women from the Nurses' Health Study II. Blood samples were collected from 1996 to 1999. The analysis includes 316 cases of breast cancer diagnosed after blood donation and before June 1, 2003, who had two controls matched on age, fasting status, time of day and month of blood collection, race/ethnicity, and timing of blood draw within the menstrual cycle. Sixty-three percent of participants provided a timed follicular and luteal menstrual phase blood sample; other women provided a single untimed sample. When including all women, we observed a positive association between prolactin and breast cancer risk [relative risk (RR), top quartile versus bottom quartile, 1.5; 95% confidence interval (95% CI), 1.0-2.3; P_{trend} = 0.03] that was slightly stronger among estrogen receptor–positive/progesterone receptor–positive tumors (comparable RR, 1.9; 95% CI, 1.1-3.3; P_{trend} = 0.04). Associations were similar among premenopausal women only. However, we did not find an association between prolactin and breast cancer risk among the subset of women who only provided timed samples (comparable RR, average of timed samples, 1.3; 95% CI, 0.8-2.3; P_{trend} = 0.40). The association seemed stronger among women ≥45 years old and for cases diagnosed within ~4 years of blood collection. Our data suggest a modest positive association between prolactin and breast cancer risk among predominately premenopausal women; however, further follow-up is needed to increase power for subgroup analyses. (Cancer Res 2006; 66(4): 2476-82)

Introduction

Prolactin is involved in the proliferation and differentiation of normal mammary epithelium and in stimulating lactation (1, 2); both *in vitro* and animal studies have suggested that prolactin can influence breast carcinogenesis (2). Prolactin can promote cell proliferation (3–5), alter cell motility (6), and increase tumor vascularization (2, 7). We reported recently that prolactin was associated with a modestly increased risk of postmenopausal breast cancer (8). The association was strongest for estrogen receptor (ER)–positive/progesterone receptor (PR)–positive breast cancers [relative risk (RR), top quartile versus bottom quartile, 1.8; P_{trend} < 0.001].

Few epidemiologic studies have examined the association of prolactin with breast cancer in younger women. Case-control studies of premenopausal breast cancer have been small and the results inconsistent (9–15). Prolactin is influenced by stress (including surgery); hence, retrospective case-control studies, in which sample collection occurs after diagnosis, are susceptible to bias (16–18). Three small prospective studies (n = 21-71 cases) did not find any significant associations between premenopausal levels of prolactin and breast cancer risk, but all had limited power (19–21).

To better understand the possible role of prolactin in breast cancer in younger women, we conducted a large prospective, nested case-control study to examine the relationship between plasma prolactin concentrations and risk of breast cancer in predominately premenopausal women from the Nurses' Health Study (NHS) II.

Materials and Methods

Study population. The NHSII was established in 1989, when 116,678 female registered nurses, ages 25 to 42 years, completed and returned a questionnaire. The NHSII cohort has been followed every 2 years since inception by questionnaire to update exposure variables and ascertain newly diagnosed disease. The racial/ethnic breakdown is 94% White, 2% Asian, 2% African American, and 2% Hispanic.

Between 1996 and 1999, 29,611 cohort members who were cancer-free provided a blood sample; participants were between 32 and 54 years old at blood collection. Women were sent a blood collection kit containing the supplies needed to have blood samples drawn by a local laboratory or colleague (e.g., needle, tourniquet, and sodium heparin blood collection tubes). Premenopausal women, who had not taken any type of hormones, been pregnant, or breast-fed in the previous 6 months (n = 18,521), provided an initial 15-mL blood sample drawn on the third to fifth day of their menstrual cycle (follicular blood draw) and a second 30-mL blood sample drawn 7 to 9 days before the anticipated start of their next cycle (luteal blood draw). We counted the timing of the luteal sample from the estimated first day of the woman's next menstrual cycle, as this generally is more accurate than counting forward from day 1 of the current cycle (22, 23). Follicular samples were placed in a refrigerator for 8 to 24 hours by the participant; then, she aliquoted the plasma into a labeled cryotube included in the blood collection kit. This plasma was frozen by the woman until the second blood collection. On the day of the luteal blood collection, the woman shipped both follicular and luteal blood samples, via overnight courier and with an ice-pack, to our laboratory where the luteal blood draw was processed, separated into plasma, RBC, and WBC components, and aliquoted into labeled cryotubes. The follicular and luteal phase blood samples hereafter are called timed blood samples.

Women who were ineligible to provide timed blood samples (i.e., perimenopausal, postmenopausal, had a simple hysterectomy, or currently

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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used oral contraceptives or other hormones) or who declined to give timed blood samples (n = 11,090) provided a single 30-mL blood sample (hereafter called an untimed sample). These samples were shipped and processed similar to the luteal timed samples. The stability of prolactin in whole blood not processed for 24 to 48 hours has been shown previously (24).

Women providing timed blood samples completed a questionnaire that recorded the first day of the menstrual cycle during which the blood samples were drawn and the dates, time of day, and number of hours since last food intake before both blood draws. These women also completed and returned a postcard recording the first day of their next menstrual cycle to determine the timing of the luteal phase blood draw; 98% of women with timed samples returned the postcard. Women providing an untimed sample completed a questionnaire that recorded the date, time of day, and number of hours since last food intake for their single blood draw. Other information, such as the participant's current weight, normal menstrual cycle length (timed samples only), recent medication use, and current smoking status, was collected for all women.

All samples have been stored in the vapor phase of continuously monitored liquid nitrogen freezers since collection. Overall, 61% of samples were collected while fasting for >8 hours and 94% of untimed and luteal timed samples were received within 26 hours of collection. NHSII women providing a blood sample were similar to the total cohort in age (mean in blood versus overall cohort in 1997) (43.2 versus 42.4 years), body mass index (BMI; 26 versus 26 kg/m^2), parity (1.9 versus 1.9 children), age at menarche (12 versus 12 years), ever smoked (34% versus 36%), ever oral contraceptive use (86% versus 88%), and >10 years oral contraceptive use (10% versus 13%). Follow-up of the blood study cohort was 98% in 2003. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Women who provided a timed sample were considered to be premenopausal. Among women providing an untimed sample, a woman was considered to be premenopausal if she (a) reported that her periods had not ceased or (b) had a hysterectomy but had at least one ovary remaining and was ≤47 (for nonsmokers) or ≤45 (for smokers) years old. A women was considered to be postmenopausal if she (a) reported that her natural menstrual periods had ceased permanently (she could be on postmenopausal hormones) or (b) had a bilateral oophorectomy. All other women were considered to be of unknown menopausal status.

Cases had no reported cancer diagnosis before blood collection and were diagnosed with breast cancer after blood collection but before June 1, 2003. In all, 319 cases of breast cancer (n = 80 *in situ*) were reported and confirmed by medical record review (n = 301) or by verbal confirmation of the diagnosis by the nurse (n = 19). Due to the high confirmation rate in medical record review (99%), these latter cases were included in the analysis. Two cases were originally control women who subsequently developed breast cancer; we have only included these women as controls. Time from blood draw to diagnosis ranged from 1 to 87 months (mean, 31 months). Cases were matched to two controls on age (±2 years), menopausal status at blood collection and diagnosis (premenopausal, postmenopausal, unknown), month and year of blood draw (±2 months), and ethnicity (African American, Asian, Hispanic, Caucasian, Other); additionally, for each blood collection, we matched on time of day (±2 hours) and fasting status (<2, 2-4, 5-7, 8-11, >12 hours). Further, cases providing timed samples were matched on the luteal day of the blood collection (date of next period minus date of luteal draw, ±1 days). For each matching variable, >90% of case-control pairs had exact matches.

Laboratory assays. Prolactin was measured using a microparticle enzyme immunoassay. Samples were assayed, in two batches, at the Reproductive Endocrinology Unit Laboratory at the Massachusetts General Hospital, using the AxSYM Immunoassay system (Abbott Diagnostics, Chicago, IL), in October 2004 and March 2005. The limit of detection was 0.6 ng/mL. Total estradiol and testosterone were measured at Quest Diagnostics (San Juan Capistrano, CA) by RIA following extraction and celite column chromatography (25).

Follicular and luteal samples from a single woman and case-control sets were assayed together. The samples were ordered randomly and labeled so that the laboratory was blinded to both case-control status and samples belonging to the same woman. In each batch, we included blinded replicate plasma samples to assess laboratory precision. The interassay coefficient of variation ranged from 4% to 12%.

Statistical analysis. We considered plasma concentrations of prolactin for all women and all premenopausal women [i.e., those with timed samples (using the average of the two phases) and those with untimed samples]. To increase the sample size, we included postmenopausal women in the primary analysis because they have similar prolactin levels as premenopausal women (median in controls, follicular, 13.4 ng/mL; luteal, 15.7 ng/mL; untimed premenopausal, 13.7 ng/mL; postmenopausal, 13.5 ng/mL) and most had only been menopausal a short time (median, 4 years). We also separately evaluated the subset of women with timed samples in the luteal and follicular phases. We also considered the average of the two menstrual phases because prolactin levels do not vary substantially by menstrual phase and to reduce random within-person error (26). In a subset of 113 women from the NHSII blood cohort, we examined the within-person stability of prolactin over a 3-year period (27). The intraclass correlation (ICC) for the luteal and follicular phases were 0.41 and 0.55, respectively, whereas the ICC for the average of the two phases was higher (0.64).

Mean plasma prolactin concentrations from a subset of 12 samples run in both batches differed by batch, indicating that there was some laboratory drift over time but were highly correlated (r = 0.99). Therefore, we used batch-specific quartile cut points. We generated quartiles based on control distributions (see Supplementary Table SA).

We excluded women who were missing prolactin values related to technical difficulties with the assay or low volume (luteal phase, n = 2 controls; follicular phase, n = 4 cases and 11 controls; untimed sample, 1 control). We identified statistical outliers based on the generalized extreme studentized deviate many-outlier detection approach (28); women with prolactin concentrations >120 ng/mL were excluded (luteal phase, n = 2 controls; follicular phase, n = 2 controls; untimed sample, n = 1 case and 1 control). Overall, 316 cases and 633 controls were available for analysis; the number of cases and controls available varies by type of blood draw and is noted in the footnotes to Table 1.

For our primary analysis, we used conditional logistic regression to estimate RRs and 95% confidence intervals (95% CI) comparing quartiles of prolactin concentrations (29). In addition, we estimated RRs and 95% CIs comparing quartiles of prolactin concentrations for various case groups (invasive only, tumor size ≤2 cm, ER+/PR+, and time between blood draw and diagnosis) using unconditional logistic regression adjusting for matching factors. We only examined ER+/PR+ tumors because we had too few cases with the other subtypes to examine separately (ER+/PR−, n = 20; ER−/PR+, n = 3; ER−/PR−, n = 34). When examining the relationship between prolactin levels and breast cancer by time between blood draw and diagnosis, we used polytomous unconditional logistic regression to determine if the RRs across case groups differed, by comparing 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 (30). Only 14 breast cancer cases who were premenopausal at blood collection (5 with timed samples) were postmenopausal at diagnosis; therefore, we were unable to examine these cases separately. Secondary, *a priori*, analyses composed of excluding women with a high prolactin level (>24 ng/mL), excluding women taking antidepressants or postmenopausal hormones, and stratifying by age (<45 versus ≥45 years), BMI at blood draw (<23, 23 to <28, ≥28 kg/m^2), or oral contraceptive use history (never/<2 versus ≥2 years use); these analyses employed unconditional logistic regression adjusting for matching factors.

All models were adjusted for the following *a priori* potential confounders: BMI at age 18 (<21, 21 to <23, ≥23 kg/m^2), weight change from age 18 to blood draw (<5, 5 to <20, ≥20 kg), family history of breast cancer (yes, no), and age at menarche (<12, 12, 13, ≥14 years). Further adjustment for breast-feeding, antidepressant use at blood draw, oophorectomy, history of benign breast disease, or duration of oral contraceptive use did not substantially alter the results. We considered separate adjustment for 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 years/≥5 children, age at

Table 1. Characteristics at blood collection of cases and their matched control subjects from the NHSII

	Case subjects (n = 316)	Control subjects (n = 633)
Age (y), mean (SD)	45.3 (4.3)	45.1 (4.3)
Parity,* mean (SD)	2.2 (0.8)	2.3 (1.0)
BMI at age 18 (kg/m^2), mean (SD)	20.9 (3.0)	21.0 (2.7)
BMI at blood draw (kg/m^2), mean (SD)	25.4 (5.3)	25.8 (6.1)
Had a follicular or luteal phase sample, %	62.3	62.6
Premenopausal at blood collection, %	75.6	75.5
Family history of breast cancer, %	16.4	10.4
History of benign breast disease, %	23.7	16.0
Took antidepressant medication, %	13.0	12.0
Age at menarche ≥14 y, %	15.8	17.5
Ever used oral contraceptives, %	84.8	86.3
Current use of postmenopausal hormones, %	18.7	17.9
	Median prolactin (10th-90th percentile)	Median prolactin (10th-90th percentile)
Follicular phase of the menstrual cycle (ng/mL)†	13.4 (7.1-27.2)	13.4 (7.3-27.3)
Luteal phase of the menstrual cycle (ng/mL)‡	16.2 (8.5-31.4)	15.7 (8.4-30.0)
Average of follicular and luteal phases (ng/mL)§	15.2 (8.9-28.5)	15.3 (8.4-26.4)
Average of timed + untimed samples (ng/mL)‖	14.9 (8.9-28.4)	14.5 (8.3-26.1)

*Among parous women only.
†193 cases and 384 controls have a sample in the follicular phase of the menstrual cycle.
‡197 cases and 393 controls have a sample in the luteal phase of the menstrual cycle.
§193 cases and 382 controls have a sample in both follicular and luteal phases of the menstrual cycle.
‖312 cases and 620 controls that have either follicular and luteal phase samples (n = 193 cases/382 controls) or an untimed sample (premenopausal, 42 cases/83 controls; postmenopausal, 65 cases/135 controls; unknown menopausal status, 12 cases/20 controls).

first birth ≥25 years/≥5 children), as this may be part of the biological pathway through which prolactin affects breast cancer (2). In a secondary analyses, we considered adjustment for follicular or luteal total estradiol (women with timed samples only) and for total testosterone (all women). Tests for trend were conducted by modeling batch-specific quartile medians of prolactin concentrations and calculating the Wald statistic (31). All Ps were based on two-sided tests and considered statistically significant if ≤0.05.

Results

Participants were 32 to 52 years old (mean, 45 years) at blood collection (Table 1). Differences between cases and controls for age at menarche, parity, and BMI at blood draw generally were small but in the expected direction. A higher percentage of cases versus controls had a family history of breast cancer (16.4% versus 10.4%, respectively) and a history of benign breast disease (23.7% versus 16.0%, respectively). Overall, cases had a slightly higher median prolactin concentration (P = 0.08); results were similar for all premenopausal women (P = 0.11). Among women with a timed sample, cases and controls had similar levels of prolactin in the follicular and luteal phases and for the average of the phases (P = 0.54, 0.56, and 0.56, respectively).

Among all women, we observed an increased risk of breast cancer in the highest versus lowest fourth of prolactin concentrations (RR, 1.5; 95% CI, 1.0-2.3; P_{trend} = 0.03) that seemed slightly stronger among ER+/PR+ tumors (RR, 1.9; 95% CI, 1.1-3.3; P_{trend} = 0.04; Table 2). Results were similar when considering all premenopausal women (RR, all cases, 1.5; 95% CI, 1.0-2.5; P_{trend} = 0.06 and RR, ER+/PR+ cases, 1.9; 95% CI, 1.0-3.7; P_{trend} = 0.10), women with an untimed sample (RR, all cases, 1.7; 95% CI, 0.9-3.3; P_{trend} = 0.14 and RR, ER+/PR+ cases, 2.7; 95% CI, 1.0-7.0; P_{trend} = 0.03), or postmenopausal women (RR, all cases, 1.5; 95% CI, 0.6-3.7; P_{trend} = 0.51). However, we observed no significant associations between plasma prolactin concentrations and risk of breast cancer among the subset of premenopausal women with a timed luteal or follicular phase sample. The results were similar when excluding women with a prolactin concentration >24 ng/mL, excluding women taking antidepressants or postmenopausal hormones at blood draw, and for cases with a tumor size <2 cm (data not shown). Further adjustment for age at first birth and parity slightly attenuated the results (data not shown). The correlations between prolactin and total estradiol or testosterone generally were low (Spearman r < 0.19); additional adjustment for these hormones did not substantially alter the risk estimates (data not shown).

In general, we observed that the relationship between prolactin and breast cancer was strongest among women with a short time between blood collection and diagnosis, although the differences were not statistically significantly different (Table 3). For example, among all premenopausal women, the RR comparing the top versus bottom fourth of prolactin measures was 1.6 (95% CI, 0.80-3.0; P_{trend} = 0.11) for cases diagnosed <2 years after blood draw, 1.8 (95% CI, 0.9-3.5; P_{trend} = 0.03) for cases diagnosed 2 to <3.875 years after blood draw, and 1.1 (95% CI, 0.5-2.3; P_{trend} = 0.63) for cases diagnosed ≥3.875 years after blood draw.

The association between prolactin and breast cancer seemed to be stronger in women ≥45 versus <45 years old. Among all women, the RR comparing the top versus bottom fourth of prolactin levels was 1.7 (95% CI, 1.0-2.9; P_{trend} = 0.05) for women ≥45 years old versus 1.2 (95% CI, 0.6-2.2; P_{trend} = 0.53) for women <45 years old. Results were similar for all premenopausal women or those with

Table 2. Multivariate RR (95% CI) of breast cancer by quartile of plasma prolactin concentrations among predominately premenopausal women

	Prolactin concentrations				P_{trend}*
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
All cases and controls†					
All cases (n = 312 cases)‡	1.0 (Reference)	1.1 (0.8-1.7)	0.9 (0.6-1.4)	1.5 (1.0-2.3)	0.03
Invasive only (n = 216 cases)§	1.0 (Reference)	1.1 (0.7-1.7)	0.9 (0.5-1.4)	1.5 (0.9-2.4)	0.04
ER+/PR+ (n = 142 cases)§	1.0 (Reference)	1.4 (0.8-2.5)	1.2 (0.7-2.2)	1.9 (1.1-3.3)	0.04
All premenopausal cases and controls†					
All cases (n = 235 cases)‡	1.0 (Reference)	1.2 (0.7-1.9)	0.9 (0.6-1.5)	1.5 (1.0-2.5)	0.06
Invasive only (n = 159 cases)§	1.0 (Reference)	1.2 (0.7-2.0)	0.9 (0.5-1.6)	1.6 (0.9-2.7)	0.09
ER+/PR+ (n = 109 cases)§	1.0 (Reference)	1.5 (0.8-3.0)	1.2 (0.6-2.5)	1.9 (1.0-3.7)	0.10
Follicular phase prolactin					
All cases (n = 193 cases)‡	1.0 (Reference)	1.0 (0.6-1.6)	0.7 (0.4-1.3)	1.3 (0.8-2.1)	0.28
Invasive only (n = 130 cases)§	1.0 (Reference)	1.1 (0.6-1.9)	0.7 (0.4-1.4)	1.3 (0.7-2.3)	0.41
ER+/PR+ (n = 89 cases)§	1.0 (Reference)	1.2 (0.6-2.3)	1.0 (0.5-2.0)	1.5 (0.7-3.1)	0.25
Luteal phase prolactin					
All cases (n = 197 cases)‡	1.0 (Reference)	0.9 (0.5-1.5)	1.0 (0.6-1.6)	1.0 (0.6-1.7)	0.79
Invasive only (n = 131 cases)§	1.0 (Reference)	0.6 (0.3-1.2)	0.8 (0.5-1.5)	0.9 (0.5-1.7)	0.85
ER+/PR+ (n = 89 cases)§	1.0 (Reference)	0.7 (0.3-1.4)	0.9 (0.4-1.7)	0.8 (0.4-1.6)	0.71
Average follicular and luteal					
All cases (n = 193 cases)‡	1.0 (Reference)	1.2 (0.7-2.0)	0.7 (0.4-1.2)	1.3 (0.8-2.3)	0.40
Invasive only (n = 130 cases)§	1.0 (Reference)	1.1 (0.6-1.9)	0.6 (0.3-1.2)	1.3 (0.7-2.4)	0.40
ER+/PR+ (n = 89 cases)§	1.0 (Reference)	1.0 (0.7-2.8)	0.9 (0.4-1.8)	1.4 (0.7-2.9)	0.49

*Determined using batch-specific quartile medians.

†For those with timed samples, we used the average of the two phases; for women with untimed samples, we used their individual sample.

‡Conditional logistic regression adjusting for BMI at age 18, weight change from age 18 to blood draw, family history of breast cancer, and age at menarche.

§Unconditional logistic regression adjusting for BMI at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, and matching factors.

timed samples and when excluding three cases who had a pregnancy after their blood collection (data not shown). We did not observe differences in risk by past oral contraceptive use or by BMI at blood draw (data not shown).

Discussion

In this study of predominately premenopausal women, we observed a positive association between plasma prolactin concentrations and breast cancer risk both among all women and all premenopausal women. However, we found few associations for the subset of premenopausal women who provided a follicular and luteal phase sample. Further, the relationship between prolactin and breast cancer tended to be stronger for ER+/PR+ tumors, among women diagnosed within a few years of blood collection, and among women ≥45 years old, although none of these differences was statistically significant.

Three previous prospective nested case-control studies have examined the relationship between prolactin and breast cancer (19–21). The initial study was among a cohort of >5,000 premenopausal women at blood collection living on the island of Guernsey, who were followed for up to 22 years (n = 71 cases); the RR comparing the top versus bottom quintiles of prolactin levels was 1.1 (95% CI, 0.5-2.2; P_{trend} = 0.36; ref. 21). The Washington County Cohort with 21 cases reported a RR comparing the top versus bottom tertile of prolactin levels of 1.1 (95% CI, 0.3-4.1; ref. 20). A third study, among Japanese women, had 46 cases; the RR for a 1-unit increment in $\log_{10}$ prolactin concentrations was 1.0 (95% CI, 0.02-47.4; ref. 19). The number of cases in these studies was very small, precluding the ability to detect even a moderate to strong association. We observed a positive association between prolactin levels and breast cancer risk among all women in our study. The association was similar for all premenopausal women (including women with timed and untimed samples), indicating that postmenopausal women and women with an unknown menopausal status were not driving the association. The magnitude of this relationship is similar to that observed among postmenopausal women in the NHS (8). The RR between the top and the bottom quartiles for all cases was 1.5 for the current study and 1.3 for the NHS study; the comparable RR for ER+/PR+ tumors was 1.9 for the current study and 1.8 for the NHS study.

Data from both animal and *in vitro* models suggest a biological basis for the role of prolactin in mammary carcinogenesis. Breast cancer cells/tissue express prolactin (32–35) and the prolactin receptor (34–37). Although normal breast tissue also expresses the prolactin receptor, levels are higher in tumor tissue (37, 38). In several mouse models, prolactin induces tumor formation (39, 40) and increases tumor growth rates (5) and the number of cells in the S phase (40). Prolactin also may lead to higher cell proliferation rates (3–5) and increased levels of cyclin D1 (3, 4) in breast cancer cell lines.

Our data also suggest that prolactin may be slightly more strongly associated with ER+/PR+ tumors; we specifically examined this tumor subtype because PR activity seems to be dependent on the ER, indicating that the tumor likely has a functional ER (41). Previous data suggest that the ER and prolactin receptor are coexpressed (36, 37, 42–45), although some smaller studies (34, 46–50) did not find a relationship. Despite these data, there is no clear mechanism underlying this coexpression. However, long-term prolactin exposure can increase ER expression in cell lines (3, 37) and constant prolactin expression in transgenic mice can lead to the development of ER+ tumors, although ER+ tumors are extremely rare in this mouse model. These data, taken together with similar findings in our previous study of postmenopausal women (8), suggest that prolactin may be important in the development of ER+ tumors.

We found that the relationship between prolactin and breast cancer risk was stronger in women ≥45 years old. This may explain why we see little or no association among premenopausal women with timed samples, as only 38% of women were >45 years old in this subset. This interaction suggests that perhaps prolactin levels in late premenopause, perimenopause, and postmenopause are more important in the development of breast cancer than levels during early premenopausal years, although prolactin levels do not vary substantially between premenopausal and postmenopausal years. To our knowledge, there are no biological data supporting this association. This interaction is not due to a different proportion of ER+/PR+ tumors in women <45 versus ≥45 years old (67.4% versus 64.3% of cases, respectively). Given the smaller number of cases when stratifying (n = 133 cases for ages <45 years and 177 cases for ages ≥45 years), these results should be interpreted with caution.

Prolactin also seemed to be more strongly associated with risk of breast cancer among cases that were diagnosed within ~4 years of blood collection. In our study of postmenopausal women, we also found that the observed association was stronger, although not statistically significantly different, for cases diagnosed within 4 years of blood collection versus >4 years (8). Interestingly, in this larger study, we still observed a statistically significant positive association for prolactin and breast cancer risk among cases diagnosed >4 years after blood collection (RR comparing the top versus bottom quartiles, ~1.5); in the current study, we had too few cases in the comparable group (n = 78) to rule out an association of this magnitude, necessitating further follow-up. It is possible that the within-woman reproducibility of prolactin levels decreases over time, thus potentially attenuating risk estimates in later years. However, breast tumors can secrete prolactin (33) and thereby may increase plasma concentrations. One study reported that immunohistochemical staining of prolactin in breast tumors was correlated with plasma prolactin concentrations (r = 0.41). Further, Lachelin et al. (51) reported that prolactin levels in some breast cancer patients with hypophysectomy had near-normal levels of prolactin within several weeks of surgery, suggesting that at least some circulating prolactin may be derived from breast tissue.

Table 3. Multivariate RR (95% CI) of breast cancer by quartile of plasma prolactin concentrations by time between blood draw and diagnosis

	Prolactin concentrations				P_{trend}*	$P_{heterogeneity}$†
	Quartile 1	Quartile 2	Quartile 3	Quartile 4		
All cases and controls‡						
<2 y (n = 122 cases)	1.0 (Reference)	1.0 (0.6-1.8)	0.7 (0.4-1.4)	1.4 (0.8-2.5)	0.13	0.18
2 to <3.875 y (n = 110 cases)	1.0 (Reference)	0.9 (0.5-1.8)	1.0 (0.5-2.0)	1.9 (1.0-3.4)	0.01	
≥3.875 y (n = 78 cases)	1.0 (Reference)	1.2 (0.6-2.5)	0.9 (0.4-1.8)	1.0 (0.4-2.1)	0.51	
All premenopausal cases and controls‡						
<2 y (n = 86 cases)	1.0 (Reference)	1.1 (0.6-2.1)	0.7 (0.3-1.5)	1.6 (0.8-3.0)	0.11	0.13
2 to <3.875 y (n = 79 cases)	1.0 (Reference)	0.9 (0.4-2.1)	1.0 (0.5-2.1)	1.8 (0.9-3.5)	0.03	
≥3.875 y (n = 70 cases)	1.0 (Reference)	1.5 (0.7-3.1)	1.3 (0.6-2.7)	1.1 (0.5-2.3)	0.63	
Follicular phase prolactin						
<2 y (n = 63 cases)	1.0 (Reference)	0.7 (0.3-1.6)	0.6 (0.2-1.4)	1.5 (0.7-3.3)	0.08	0.20
2 to <3.875 y (n = 64 cases)	1.0 (Reference)	1.0 (0.4-2.2)	1.0 (0.5-2.4)	1.5 (0.7-3.4)	0.25	
≥3.875 y (n = 66 cases)	1.0 (Reference)	1.1 (0.5-2.3)	0.6 (0.3-1.5)	0.8 (0.3-1.9)	0.46	
Luteal phase prolactin						
<2 y (n = 63 cases)	1.0 (Reference)	0.8 (0.3-2.0)	1.3 (0.6-3.0)	1.7 (0.8-3.7)	0.27	0.38
2 to <3.875 y (n = 66 cases)	1.0 (Reference)	0.7 (0.3-1.7)	0.9 (0.4-2.0)	1.1 (0.5-2.4)	0.74	
≥3.875 y (n = 68 cases)	1.0 (Reference)	0.9 (0.4-1.8)	0.8 (0.4-1.6)	0.6 (0.2-1.2)	0.33	
Average follicular and luteal						
<2 y (n = 63 cases)	1.0 (Reference)	1.0 (0.5-2.4)	0.7 (0.3-1.8)	2.1 (0.9-4.7)	0.08	0.10
2 to <3.875 y (n = 64 cases)	1.0 (Reference)	1.1 (0.5-2.4)	0.6 (0.3-1.5)	1.4 (0.6-3.1)	0.49	
≥3.875 y (n = 66 cases)	1.0 (Reference)	1.3 (0.6-2.6)	0.7 (0.3-1.6)	0.7 (0.3-1.7)	0.44	

NOTE: Adjusted for BMI at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, and matching factors.
*Determined using batch-specific quartile medians.
†Determined using polytomous logistic regression and the likelihood ratio test, comparing a model constraining RRs to be the same across all case groups versus a model allowing the RRs to differ across case groups.
‡For those with timed samples, we used the average of the two phases; for women with untimed samples, we used their individual sample.

This study has several limitations. First, several forms of prolactin circulate in human plasma, which may have different biological activities (52, 53); further, a prolactin-binding protein has been identified in plasma that may alter prolactin activity (54), which we could not account for here. The immunoassay used in this study measures most prolactin isoforms (55); however, it cannot distinguish between them or determine what amount of prolactin is bound to the binding protein. Second, there was laboratory drift over time, despite the use of a high-precision assay, which precluded us from considering the relationship of absolute prolactin concentrations with breast cancer. Third, prolactin has a strong circadian rhythm (56) and increases after a noontime meal (57). To minimize misclassification related to time of day of blood draw and fasting status, we closely matched cases and controls on both of these factors. Further, we evaluated circulating levels of prolactin, which are an indirect marker of exposure at the breast especially because data suggest that there is extrapituitary prolactin production in the breast (2).

This study also has several strengths. This is the largest prospective study of prolactin concentrations and breast cancer risk in predominately premenopausal women, providing more power to detect an association than previous studies. Despite this, we had limited power to detect associations in subgroup analyses. Another strength of this study was that for most premenopausal women we were able to obtain timed follicular and luteal blood samples, which allowed us to examine relationships with both phases of the menstrual cycle separately.

Recent evidence has suggested that high prolactin concentrations after menopause may increase risk of invasive and ER+/PR+ postmenopausal breast cancer (8). We found a similar relationship among predominately premenopausal women in this study. However, this association may be confined to women >45 years old or to those diagnosed shortly after blood collection. Further follow-up in this cohort, along with assessments in other prospective studies, will be necessary to better define the relationship between prolactin and breast cancer risk in younger women.

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