Research Article

Cancer Epidemiology, Biomarkers & Prevention

Prolactin Determinants in Healthy Women: A Large Cross-Sectional Study within the EPIC Cohort

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Abstract

Background: Experimental and epidemiologic data suggest that higher circulating prolactin is associated with breast cancer risk; however, how various risk factors for breast cancer influence prolactin levels in healthy women is not clear.

Methods: We analyzed cross-sectional associations between several suggested reproductive and lifestyle risk factors for breast cancer and circulating prolactin among pre- and postmenopausal women, taking into account the use of current postmenopausal hormone therapy, among 2,560 controls from a breast cancer nested case–control study within the EPIC cohort.

Results: Adjusted geometric mean prolactin levels were significantly higher among premenopausal women, and among postmenopausal women using hormone therapy compared with nonusers (8.2, 7.0, and 6.3 ng/mL, respectively; $P_{cat} = <0.0001$). Furthermore, prolactin levels were significantly higher among users of combined estrogen–progestin hormone therapy compared with users of estrogen-alone hormone therapy (6.66 vs. 5.90 ng/mL; $P_{cat} = 0.001$). Prolactin levels were lower among parous women compared with nulliparous women (8.61 vs. 10.95 ng/mL; $P_{cat} = 0.0002$, premenopausal women); the magnitude of this difference depended on the number of full-term pregnancies (22.1% lower, ≥3 vs. 1 pregnancy, $P_{trend} = 0.01$). Results for parity were similar but lower in magnitude among postmenopausal women. Prolactin did not vary by other studied factors, with the exception of lower levels among postmenopausal smokers compared with never smokers.

Conclusions: Our study shows that current hormone therapy use, especially the use of combined hormone therapy, is associated with higher circulating prolactin levels in postmenopausal women, and confirms prior findings of lower circulating prolactin in parous women.

Impact: Our study extends the knowledge linking various breast cancer risk factors with circulating prolactin. *Cancer Epidemiol Biomarkers Prev; 23(11); 2532–42. ©2014 AACR.*

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Introduction

Prolactin is a polypeptide hormone mainly implicated in growth and differentiation of breast epithelial cells during different stages of the reproductive life history of women (1). It is synthesized primarily by the lactotrophs of the anterior pituitary and the secretion of prolactin is suggested to be controlled by various prolactin-releasing factors (including estrogen and progesterone) and prolactin-inhibiting factors (dopamine; refs. 1, 2).

Recent epidemiologic data suggest that higher circulating prolactin levels are associated with increased risk of breast cancer (3–5), and several potential breast cancer risk factors have been examined in relation to circulating prolactin levels (6–9), although these latter results are inconsistent. Regarding reproductive factors, it is well known that women who have experienced a full-term pregnancy (FTP) have lower levels of prolactin compared with women who have not been pregnant (6, 10, 11). The impact of other factors such as the number of FTPs, breastfeeding, previous use of oral contraceptives, age at and years since menopause, lifestyle factors, and adiposity indices on circulating prolactin levels is less clear. Furthermore, results regarding the effect of postmenopausal hormone therapy use on the blood prolactin levels are inconsistent.

We therefore investigated in a comprehensive manner the cross-sectional associations between several reproductive and lifestyle risk factors for breast cancer and circulating levels of prolactin in healthy pre- and postmenopausal women, taking into account the use of postmenopausal hormone therapy (HT) at the time of blood donation. The study population consisted of control subjects from a nested case–control study on breast cancer and prolactin levels within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

Materials and Methods

Study population

The EPIC is a multicenter cohort study designed to investigate the relationships between diet, metabolic factors and cancer, consisting of approximately 360,000 women and 150,000 men aged mostly between 25 and 70 years. All participants were enrolled between 1992 and 2000 from 23 centers in 10 European countries: Denmark, France, Germany, Greece, Italy, Norway, Spain, Sweden, the Netherlands, and the United Kingdom. Extensive details about the cohort population and data collection procedures at baseline are given elsewhere (12). Briefly, participants provided questionnaire information, covering dietary habits and other lifestyle factors, history of previous illness, and invasive surgical operations. Women provided detailed information about their menstrual cycle, menopausal status, reproductive history, and use of oral contraceptives and postmenopausal hormone therapy. In addition to the questionnaire assessments, measurements of height, weight, and waist and hip circumferences were performed according to standardized protocols, and approximately two thirds of study participants also provided a blood sample for the laboratory analyses (12).

Selection of subjects

Women included in this analysis were selected as control subjects in a large case–control study on breast cancer and prediagnostic prolactin levels nested within the EPIC cohort ($n = 2{,}578$). Eligible were women who could be clearly classified as being either premenopausal or postmenopausal at the time of blood donation, did not have any previous diagnosis of cancer (except nonmelanoma skin cancer), and had given information on postmenopausal exogenous hormone use at blood donation. Sweden was not included in the analysis because of incomplete data on exogenous hormone use. One woman with a prolactin value above the detection limit of the assay and 17 women with missing prolactin values were excluded from the study, leaving a total of 2,560 subjects for analyses.

Menopausal status and HT use at blood collection

Women who were $\leq$42 years of age or reported having had at least 9 menstrual periods in the last 12 months were classified as premenopausal. Women were classified as postmenopausal when they reported not having any menses over the past 12 months, when older than 55 years of age (if the menstrual cycle history was missing), or when reporting bilateral ovariectomy. Women aged 42 years or older whose questionnaire data on menopausal status was incomplete were not included to the present study.

Information on postmenopausal exogenous hormone use was derived from country-specific baseline questionnaires covering never, previous, and current use of hormone therapy together with the brand name and duration of use. Current users of hormone therapy were subgrouped according to therapy regimens as estrogen alone users and combined estrogen–progestin users (estrogen with continuous or sequential daily use of progestin).

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Classification of variables

Reproductive factors of interest included in this study were age at menarche (<12, 12–14, ≥15 years), parity (never vs. ever), number of full-term pregnancies (FTP, sum of live and stillbirths, 1, 2 ≥3), age at first FTP (<25 vs. ≥25), years since first FTP (<20, 20–24, 25–29, 30–34, ≥35 years), years since last FTP (<15, 15–19, 20–24, 25–29, ≥30 years), breastfeeding (yes vs. no), duration of first breastfeeding [never (parous), 0.1–3, 4–8, ≥9 months], cumulative duration of breastfeeding (number of pregnancies multiplied by the mean of each breastfeeding duration, <1, 1–3, 4–8, 9–12, ≥13 months), previous use of oral contraceptives (never vs. previous), previous duration of oral contraceptives (<5, 5–9, ≥10 years), age at menopause (<45, 45–49, 50–54, ≥55 years), years since menopause (<5, 5–9, ≥10 years), previous use of hormone therapy (never vs. previous), duration of hormone therapy (previous and current, <1, 1–3, ≥4 years), and type of hormone therapy (estrogen alone, combined estrogen–progestin). Lifestyle and anthropometric factors of interest included smoking status (never, former, current), lifetime mean alcohol use (<6, 6–11, 12–23, ≥24 g/day), alcohol use at recruitment (0, 0.1–5, 6–10, 11–15, ≥16 g/day), a physical activity score (inactive, moderately inactive, moderately active, active), practice of vigorous physical activity (yes vs. no), body mass index, (BMI, <25, 25–29, ≥30 kg/m^2), waist circumference (<72, 72–77, 78–85, ≥86 cm), hip circumference (<95, 95–99, 100–104, ≥105 cm), body weight (<57, 57–62, 63–70, ≥71 kg), and height (<157, 157–160, 161–165, ≥166.0 cm).

Laboratory measurements

The prolactin analyses were performed in the laboratory of the Division of Cancer Epidemiology at the German Cancer Research Centre (DKFZ) and determined by immunoradiometric assay [IRMA (CT)]. All samples were analyzed at the same timeframe. The same quality controls were included in each analytic batch and laboratory personnel were blinded to the status of samples and quality controls. The detection range of the assay was 0.35 to 133 ng/mL. The mean inter- and intra-assay coefficients of variation were 4.62% and 2.17%, respectively.

Statistical analyses

All statistical analyses were conducted separately for 3 groups: premenopausal women, postmenopausal women using hormone therapy, and not using hormone therapy at the time of blood donation. Levels of prolactin were logarithmically transformed to normalize the distribution. Partial correlations between prolactin and age at blood donation, years since menopause, and endogenous hormones (e.g., logarithmically transformed estrogen, testosterone, progesterone, DHEAS, SHBG, IGFI) were calculated using the Spearman correlation coefficient. To examine the effect of age at prolactin measurement, a linear regression model with separate slopes and intercepts for pre- and postmenopausal women was used.

Adjusted geometric mean prolactin levels by categories of various factors, together with their 95% confidence intervals (95% CI), were calculated using linear regression. All analyses were adjusted for recruitment center, age (continuous), and time at blood donation (before 10 am, 10 am–1 pm, after 1 pm), fasting status (<3, 3–6, >6 hours), smoking status (never, former, current, unknown), and parity (never, ever). Years since first and last FTP were additionally adjusted for number of FTPs, and in postmenopausal women, adjustments for years since menopause were performed for age-related factors (age at blood donation, age at menopause). Adjustments for other factors such as phase of menstrual cycle in premenopausal women and BMI did not alter the results and were not included to the final model. The percent difference in the adjusted geometric means for the highest versus lowest category of established significant factors was calculated as $(e^b - 1) \times 100$. Difference of adjusted geometric mean among exposure categories (P_{cat}) was assessed by the type III test using F statistics obtained from linear regression models. Linear trends (P_{trend}) between prolactin levels and quantitative variables were tested by fitting linear regression models with continuous exposure variables. Most of the studied factors had unknown values for some women (usually less than 2%, except "age at menopause" and "years since menopause" among women who used currently hormone therapy where nearly 50% of the data were missing) and those individuals were excluded from the analyses in relation to these factors. The precise number of missing data for each studied factor can be calculated on the basis of the information provided in columns labeled "*n* (%)" in each table. All statistical tests were two sided, and $P < 0.05$ was considered statistically significant. Statistical analyses were conducted using SAS software, version 9.2 (SAS Institute).

Ethical approval

All EPIC study participants provided written consent at the time of recruitment to participate in the study. The study was approved by local ethics committees in participating countries/study centers and by the institutional review board of the International Agency for Research on Cancer (IARC, Lyon, France).

Results

The study population consisted in total of 2,560 healthy control subjects, of which 596 were classified as premenopausal and 1,964 as postmenopausal, including 900 postmenopausal women who were using hormone therapy at the time of blood donation (Table 1). Age at menarche, ever having had a FTP, and age at first FTP were similar between these groups. Postmenopausal women who were using hormone therapy were slightly younger, had lower BMI, lower waist circumference, and higher lifetime mean alcohol consumption compared with the postmenopausal women who did not use hormone therapy. Moreover, a higher percentage of hormone therapy users were previous users of oral contraceptives

Table 1. Baseline characteristics of the study population (data presented as median and range or *n* and %)

		Postmenopausal women ($n = 1964$)	
	Premenopausal women ($n = 596$)	No hormone therapy use ($n = 1,064$)	Current hormone therapy use ($n = 900$)
Age at blood collection, y	46.1 (26.7–57.1)	59.2 (41.2–76.8)	57.4 (43.1–73.6)
Age at menarche, y	13.0 (9.0–19.0)	13.0 (9.0–19.0)	13.0 (8.0–18.0)
Ever full time pregnancy	510 (86.7)	918 (86.8)	771 (88.1)
Age at first FTP, y[a]	25.0 (17.0–42.0)	25.0 (16.0–40.0)	24.0 (15.0–41.0)
Number of FTPs[a]			
1	105 (20.7)	144 (15.8)	137 (17.8)
2	283 (55.7)	411 (45.0)	372 (48.3)
≥3	120 (23.6)	359 (39.2)	261 (33.9)
Ever breastfeeding[a]	430 (85.2)	758 (84.6)	630 (83.3)
Duration of breastfeeding, all pregnancies (months)[a,b]	6.5 (0.2–89.5)	6.5 (0.2–71.6)	5.5 (0.2–97.0)
Previous oral contraceptive use	384 (64.4)	402 (38.0)	568 (63.5)
Previous duration of oral contraceptive use, y[c]	4.0 (0.5–25.0)	5.0 (1.0–25.0)	7.0 (1.0–25.0)
Age at menopause, y	—	50.0 (21.0–62.0)	49.0 (22.0–63.0)
Years since menopause	—	9.4 (0.6–46.7)	9.1 (0.4–38.2)
BMI, kg/m^2	23.9 (16.8–46.3)	25.1 (16.0–45.9)	23.9 (16.8–39.3)
Waist circumference, cm	76.1 (59.5–116.4)	80.0 (58.0–133.0)	77.0 (56.5–114.8)
Currently smoking	129 (21.6)	201 (18.9)	158 (17.6)
Lifetime mean alcohol use, g/day	4.2 (0.0–50.3)	3.7 (0.0–60.7)	5.9 (0.0–119.2)
Prolactin geometric mean, ng/mL (95% CI)[d]	8.2 (7.6–8.8)	6.3 (5.9–6.7)	7.0 (6.5–7.4)

[a]In parous women only.

[b]In women who breastfed only.

[c]In women who previously used oral contraceptives.

[d]Adjusted for center, age (continuous), time at blood donation (before 10 am, 10 am–1 pm, after 1 pm), fasting status (<3, 3–6, >6 hours), smoking (never, former, current, unknown), and parity (never, ever); *P* values from *t* test comparing pre- versus postmenopausal women ($P_{cat} \leq 0.0001$) and postmenopausal hormone therapy users versus nonusers ($P_{cat} \leq 0.0001$).

with a longer median duration of use compared with nonusers of hormone therapy. Geometric mean prolactin levels were significantly higher in premenopausal women and in women who were using postmenopausal hormone therapy compared with women who did not currently use hormone therapy (adjusted geometric means 8.2, 7.0, and 6.3 ng/mL, respectively; $P_{cat} \leq 0.0001$ for premenopausal vs. postmenopausal women, and $P_{cat} \leq 0.0001$ for postmenopausal hormone therapy users vs. nonusers, Table 1; Fig. 1).

In analysis evaluating associations between prolactin levels and characteristics at the time of blood donation (Supplementary Table S1), circulating levels of prolactin were generally highest in blood taken before 10 am and lowest around mid-day (10 am–1 pm), regardless of menopausal status and postmenopausal hormone therapy use. Among postmenopausal women, prolactin levels decreased with increasing age; however, this effect was eliminated when controlling for years since menopause in our models. Moreover, postmenopausal women who were current smokers had up to 20% lower levels of prolactin compared with never smokers. In premenopausal women, prolactin levels were not significantly associated with age at blood donation, fasting, and smoking status but varied modestly, depending on the phase of the menstrual cycle, with the highest levels in the ovulatory and luteal phase of the cycle. This variation, however, was not statistically significant ($P_{cat} = 0.16$).

With regard to reproductive factors in all three groups, parous women had significantly lower prolactin levels compared with nulliparous women (Table 2). This effect was much greater in premenopausal women (geometric mean prolactin levels comparing parous vs. nulliparous women: 8.61 vs. 10.95 ng/mL; $P_{cat} = 0.0002$) than in postmenopausal women with or without hormone therapy (geometric mean prolactin levels comparing parous vs. nulliparous women: 6.24 vs. 7.07 ng/mL, $P_{cat} = 0.01$ among hormone therapy users, and 5.42 vs. 5.89 ng/mL, $P_{cat} = 0.04$ among hormone therapy nonusers, respectively). Moreover, a higher number of FTPs was significantly associated with lower levels of prolactin (prolactin levels comparing ≥3 vs. 1 FTP; 7.77 vs. 9.49 ng/mL, $P_{trend} = 0.01$ among premenopausal women; and 5.91 vs. 6.58 ng/mL, $P_{trend} = 0.01$ among postmenopausal women using hormone therapy), although the trend test was not significant for postmenopausal women without hormone therapy (prolactin levels comparing ≥3 vs. 1 FTP, 5.55 vs. 6.02 ng/mL, $P_{trend} = 0.38$).

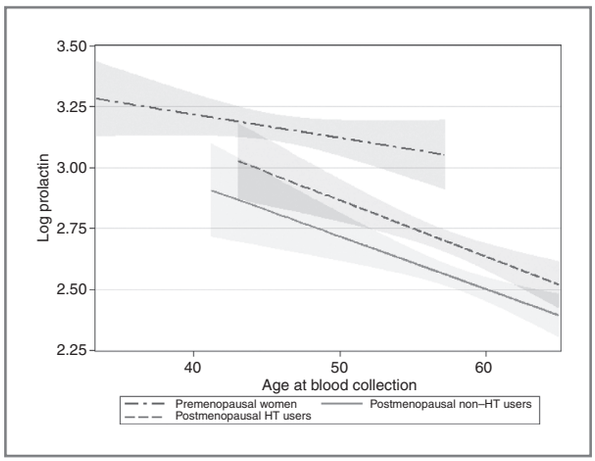

Figure 1. Log-transformed prolactin levels depending on age and menopausal status at the time of blood donation. Correlation coefficients between circulating prolactin levels and age at blood donation are 0.03 (−0.06, 0.11), $P = 0.50$ for premenopausal women; −0.12 (−0.18, −0.06), $P < 0.01$ for postmenopausal non-hormone therapy users; and −0.14 (−0.20, −0.07), $P < 0.01$ for postmenopausal hormone therapy users.

Prolactin levels did not vary significantly by age at menarche, ages at and years since first or last FTP, ever breastfeeding, duration of breastfeeding or previous use and duration of oral contraceptives in either premenopausal or postmenopausal women.

Regarding postmenopausal characteristics (Table 3), higher age at menopause and fewer years since menopause were associated with slightly higher levels of prolactin; however, after mutual adjustment these associations were both eliminated. A tendency for higher levels of prolactin with increasing duration of hormone therapy use was also found. In subgroup analyses stratified by the type of hormone therapy regimen, circulating prolactin levels were significantly higher among women who currently used combined estrogen–progestin therapy compared with the women who used estrogens alone (geometric mean prolactin levels 6.66 and 5.90 ng/mL, $P_{cat} = 0.001$ Table 3; Fig. 2).

Prolactin levels were generally not associated with anthropometric and lifestyle factors, except among women using hormone therapy, where inverse trends of circulating prolactin levels with BMI ($P_{trend} = 0.06$), waist circumference ($P_{trend} = 0.08$), body weight ($P_{trend} = 0.01$), body height ($P_{trend} = 0.09$), and hip circumference ($P_{trend} = 0.002$) were found (Supplementary Table S2). No significant variation with lifetime mean and baseline alcohol consumption and various physical activity categories were observed in any of the groups (Supplementary Table S3).

All correlations between prolactin and other endogenous hormones such as estradiol, calculated free estradiol, estrone, progesterone, testosterone, free testosterone, DHEAS, SHBG, C-peptide, and IGFI were lower than 0.14 among pre- and postmenopausal women (data not shown).

Discussion

In this large cross-sectional study, we observed that postmenopausal women who used hormone therapy at the time of blood donation had significantly higher levels of circulating prolactin compared with women who did not currently use hormone therapy. However, these higher levels seemed to be confined to those postmenopausal women who used combined estrogen–progestin therapy, and not to women who used estrogens alone. Regardless of the hormone therapy use (yes or no, combined or estrogen-only), postmenopausal women overall had lower levels of prolactin than premenopausal women. Our results also showed lower circulating prolactin levels among parous women compared with nulliparous women in all groups, although this effect was much greater in premenopausal women (27.2% lower levels) than in postmenopausal women with or without hormone therapy (13.2% and 8.6% lower levels, respectively). Furthermore, the magnitude of this reduction was associated with the number of FTPs. In contrast, prolactin levels did not vary by age at menarche, ages at or years since first and last FTP, breastfeeding, previous use, and duration of oral contraceptives and lifestyle or anthropometric factors, and were not correlated with other endogenous hormones.

Our finding of a higher level of circulating prolactin among postmenopausal women who were currently using hormone therapy compared with the women who did not use hormone therapy is generally in line with findings from a few other large studies (3, 13). However, whether these higher levels of prolactin depend on the composition/type of the used hormone therapy regimen is less clear. Our results are consistent with studies showing elevated levels of prolactin in postmenopausal women who used combined estrogen–progestin therapy (13–15), although some studies showed no significant effect of combined hormone therapy on blood prolactin levels (16–18). Likewise, in other studies the use of estrogens alone has shown to either increase (19, 20) or not to have any significant influence on circulating prolactin levels (16, 21, 22). In contrast with the postmenopausal hormone therapy use, prolactin levels are shown not to be largely influenced by oral contraceptive use in healthy premenopausal women (23).

Despite inconsistent results with regard to the various hormone therapy regimens on the circulating prolactin levels in women, it has been long proposed that estrogens stimulate prolactin secretion. However, the pattern of estrogen, progesterone, and prolactin changes during puberty (24), the menstrual cycle (25), pregnancy (26), breastfeeding (8), and the transition into menopause (25) provides evidence for a joint role of estrogens and progesterone in regulating the pituitary synthesis and release of prolactin (27). Despite hypothesized associations between prolactin and other hormones (28–30), we and others (31, 32) did not observe correlations between prolactin and endogenous hormones (i.e., estradiol and

Table 2. Adjusted[a] geometric mean prolactin levels, ng/mL (95% CI) depending on the reproductive factors

	Premenopausal women ($n = 568$)			Postmenopausal women ($n = 1,896$)		
Characteristics	***n* (%)[b]**		***n* (%)[b]**	**No hormone therapy use ($n = 1,041$)**	***n* (%)[b]**	**Current hormone therapy use ($n = 855$)**
Age at menarche, y						
<12	95 (16.7)	10.05 (8.6–11.8)	127 (12.2)	5.94 (5.2–6.7)	128 (15.0)	6.36 (5.6–7.3)
12–14	398 (70.1)	10.00 (8.8–11.4)	690 (66.3)	5.63 (5.1–6.2)	554 (64.8)	6.67 (6.0–7.5)
≥15	71 (12.5)	8.76 (7.4–10.3)	208 (20.0)	5.51 (4.9–6.2)	164 (19.2)	6.87 (6.0–7.8)
P_{cat}[f] (P_{trend})[g]		0.11 (0.41)		0.32 (0.17)		0.40 (0.50)
Parity						
Never	71 (12.4)	10.95 (9.3–12.9)	136 (13.1)	5.89 (5.2–6.6)	101 (11.5)	7.07 (6.2–8.1)
Ever	493 (86.3)	8.61 (7.6–9.7)	900 (86.5)	5.42 (4.9–6.0)	752 (85.6)	6.24 (5.6–6.9)
% Difference (ever vs. never)		27.2%		8.6%		13.2%
P_{cat}[f]		0.0002		0.04		0.01
Number of FTPs[c]						
1	105 (21.3)	9.49 (8.2–11.0)	142 (15.8)	6.02 (5.3–6.8)	136 (18.1)	6.58 (5.8–7.4)
2	274 (55.6)	8.70 (7.7–9.8)	404 (44.9)	5.38 (4.8–6.0)	359 (47.7)	6.37 (5.7–7.1)
≥3	114 (23.1)	7.77 (6.7–9.0)	354 (39.3)	5.55 (5.0–6.2)	257 (34.2)	5.91 (5.3–6.6)
% Difference (≥3 vs. 1)		22.1%[h]		8.3%		11.4%
P_{cat}[f] (P_{trend})[g]		0.01 (0.01)		0.03 (0.38)		0.05 (0.01)
Age at first FTP, y[c]						
<25	241 (48.9)	8.58 (7.5–9.8)	419 (46.6)	5.60 (5.0–6.2)	426 (56.6)	6.13 (5.5–6.8)
≥25	250 (50.7)	8.70 (7.7–9.9)	479 (53.2)	5.47 (4.9–6.1)	322 (42.8)	6.37 (5.7–7.1)
P_{cat}[f] (P_{trend})[g]		0.76 (0.54)		0.44 (0.85)		0.26 (0.34)
Years since first FTP[c]						
<20	191 (38.7)	8.47 (7.4–9.7)	10 (1.1)	5.28 (3.9–7.1)	20 (2.7)	6.96 (5.5–8.7)
20–24	158 (32.0)	9.01 (7.9–10.3)	52 (5.8)	5.93 (5.0–7.0)	47 (6.3)	5.84 (5.0–6.9)
25–29	117 (23.7)	8.43 (7.2–9.8)	155 (17.2)	5.59 (4.9–6.3)	141 (18.8)	6.11 (5.4–7.0)
30–34	23 (4.7)	8.03 (6.3–10.3)	282 (31.3)	5.46 (4.9–6.1)	248 (33)	6.44 (5.8–7.2)
≥35	2 (0.4)	10.7 (5.3–21.7)	398 (44.2)	5.52 (5.0–6.2)	290 (38.6)	6.25 (5.6–7.0)
P_{cat}[f] (P_{trend})[g]		0.62 (0.95)		0.81 (0.62)		0.48 (0.81)
Years since last FTP[c]						
<15	179 (36.3)	8.07 (7.0–9.3)	6 (0.7)	5.76 (4.0–8.4)	19 (2.5)	6.59 (5.2–8.4)
15–19	145 (29.4)	9.16 (8.0–10.5)	55 (6.1)	5.57 (4.8–6.5)	45 (6.0)	6.52 (5.5–7.7)
20–24	122 (24.7)	9.19 (7.9–10.7)	150 (16.7)	5.55 (4.9–6.3)	139 (18.5)	6.12 (5.4–6.9)
25–29	39 (7.9)	8.35 (6.8–10.2)	293 (32.6)	5.57 (5.0–6.2)	220 (29.3)	6.22 (5.5–7.0)
≥30	6 (1.2)	8.60 (5.6–13.2)	393 (43.7)	5.51 (4.9–6.1)	323 (43.0)	6.31 (5.6.7.1)
P_{cat}[f] (P_{trend})[g]		0.19 (0.22)		0.99 (0.82)		0.89 (0.63)
Breastfeeding[c]						
No	73 (14.8)	8.80 (7.5–10.4)	137 (15.2)	5.37 (4.7–6.1)	123 (16.4)	6.26 (5.5–7.1)
Yes	417 (84.6)	8.62 (7.6–9.7)	746 (82.9)	5.59 (5.0–6.2)	618 (82.2)	6.22 (5.6–6.9)
P_{cat}[f]		0.73		0.35		0.88
Duration of first breastfeeding, mo[c,d]						
Never (parous)	100 (20.3)	8.43 (7.3–9.8)	204 (22.6)	5.44 (4.9–6.1)	175 (23.3)	6.38 (5.7–7.2)
0.1–3	222 (45.0)	8.53 (7.5–9.7)	394 (43.7)	5.55 (5.0–6.2)	371 (49.3)	6.33 (5.7–7.1)
4–8	137 (27.8)	8.09 (7.0–9.3)	239 (26.5)	5.64 (5.1–6.3)	159 (21.1)	6.08 (5.4–6.9)
≥9	34 (6.9)	8.88 (7.3–10.8)	63 (7.0)	5.17 (4.5–5.9)	46 (6.1)	6.95 (5.9–8.2)
P_{cat}[f] (P_{trend})[g]		0.69 (0.95)		0.53 (0.69)		0.38 (0.71)
Duration of breastfeeding, all pregnancies, mo[c,d]						
<4	153 (36.9)	9.28 (8.0–10.7)	249 (33.6)	5.92 (5.2–6.7)	244 (40.3)	6.45 (5.8–7.2)
4–8	134 (32.3)	8.73 (7.5–10.2)	228 (30.8)	5.66 (5.0–6.4)	185 (30.5)	6.01 (5.4–6.7)

(Continued on the following page)

Table 2. Adjusted[a] geometric mean prolactin levels, ng/mL (95% CI) depending on the reproductive factors (Cont'd)

	Premenopausal women ($n = 568$)			Postmenopausal women ($n = 1,896$)		
Characteristics	***n* (%)[b]**		***n* (%)[b]**	**No hormone therapy use ($n = 1,041$)**	***n* (%)[b]**	**Current hormone therapy use ($n = 855$)**
9–12	50 (12.0)	8.39 (7.0–10.0)	90 (12.2)	5.55 (4.8–6.4)	74 (12.2)	6.78 (5.9–7.8)
≥13	78 (18.8)	8.21 (7.0–9.7)	173 (23.4)	5.51 (4.9–6.2)	103 (17.0)	5.91 (5.2–6.7)
P_{cat}[f] (P_{trend})[g]		0.30 (0.16)		0.44 (0.45)		0.10 (0.30)
Previous oral contraceptive use						
Never	202 (35.6)	9.45 (8.2–10.9)	640 (61.5)	5.66 (5.1–6.3)	313 (36.6)	6.55 (5.8–7.3)
Previous	366 (64.4)	9.91 (8.7–11.3)	394 (37.8)	5.43 (4.8–6.1)	537 (62.8)	6.66 (5.9–7.5)
P_{cat}[f]		0.31		0.18		0.62
Previous duration of oral contraceptive, y[e]						
<5	195 (53.3)	10.47 (8.8–12.5)	174 (44.2)	4.98 (4.2–6.0)	187 (34.8)	6.31 (5.5–7.3)
5–9	86 (23.5)	9.96 (8.2–12.1)	66 (16.8)	4.89 (4.0–6.0)	88 (16.4)	6.69 (5.7–7.9)
≥10	63 (17.2)	10.17 (8.3–12.5)	119 (30.2)	5.17 (4.3–6.3)	203 (37.8)	6.70 (5.8–7.8)
P_{cat}[f] (P_{trend})[g]		0.77 (0.38)		0.68 (0.27)		0.42 (0.41)

[a]Adjusted for center, age (continuous), time at blood donation (before 10 am, 10 am–1 pm, after 1 pm), fasting (<3, 3–6, >6 hours), smoking (never, former, current, unknown), and parity (never, ever) where appropriate; years since first and last FTP additionally adjusted for number of FTP; EPIC center Bilthoven (12 women) was excluded for parity and breastfeeding-related analyses because of missing information.

[b]The percentages might not add up to 100% because of missing values.

[c]In parous women only.

[d]In women who breastfed only.

[e]In women who previously used oral contraceptives.

[f]Difference of adjusted geometric means among exposure categories (P_{cat}) was assessed by the type III test using F statistics obtained from linear regression models.

[g]Linear trends (P_{trend}) between prolactin levels and quantitative variables were tested by fitting linear regression models with continuous exposure variables.

[h]$P < 0.05$.

progesterone). The mechanisms behind prolactin surges are poorly understood but it has been proposed that in addition to the direct action of steroids on the lactotrophs, steroid-induced reduced dopaminergic activity likely also increases prolactin release (28, 33).

Recent prospective studies show that higher circulating prolactin levels are associated with an increased risk of breast cancer among postmenopausal women (3–5). Given the relatively large number of women with history of hormone therapy use and the association of prediagnostic prolactin levels with subsequent breast cancer risk more pronounced among women using hormone therapy (5), the current study has an importance also from a clinical perspective. However, further studies are needed to evaluate the effects of dose, exact formulation, specific mode of administration, and duration of hormone therapy on circulating prolactin levels (34–36).

Regarding reproductive factors, in line with numerous previous reports, we found that prolactin levels among parous women were lower than those among nulliparous women (6, 9–11). Furthermore, the observed gradual decrease in prolactin levels with increasing number of FTP is in agreement with some studies (11, 37), but not with other studies, where the parity related decrease was only related to the first FTP (6, 9, 10). Mechanisms of the reduced prolactin secretion after pregnancy are currently still unclear. Possibly, long-term changes in secretion and metabolism of estrogens together with suggested increased endogenous dopaminergic activity following pregnancy may help to account for the reduction in prolactin secretion in parous women (38–40). Furthermore, consistent with previous study, we also found that parity mediated variations in prolactin levels were more pronounced in premenopausal women compared with postmenopausal women (6). As prolactin declines after menopause (6, 16, 25), this most likely also reduces the difference observed in prolactin levels between nulliparous and parous postmenopausal women (6).

As observed in numerous previous studies, ages at which women had their first or last FTP and time passed since deliveries were not associated with the variation in circulating prolactin levels (6, 8, 10, 11, 39), indicating permanent alterations in the postpregnancy prolactin response which do not depend on ages at births. With

Table 3. Adjusted[a] geometric mean prolactin levels, ng/mL (95% CI) depending on menopausal factors and current hormone therapy use in postmenopausal women

Characteristics	*n* (%)[b]	No hormone therapy use (*n* = 1,041)	*n* (%)[b]	Current hormone therapy use (*n* = 855)
Age at menopause, y				
<45	123 (11.8)	5.23 (4.5–6.0)	65 (7.6)	5.99 (4.9–7.3)
45–49	287 (27.6)	5.79 (5.1–6.6)	156 (18.2)	6.66 (5.7–7.8)
50–54	433 (41.6)	5.69 (5.0–6.5)	159 (18.6)	6.86 (5.8–8.1)
≥55	92 (8.8)	6.31 (5.4–7.4)	42 (4.9)	6.76 (5.5–8.4)
% Difference (≥55 vs. <45 years)		−17.2%[g]		−11.4%
P_{cat}[e] (P_{trend})[f]		0.03 (0.01)		0.32 (0.24)
P adjusted for years since menopause		0.18 (0.13)		0.70 (0.40)
Years since menopause				
<5	207 (19.9)	6.09 (5.3–7.0)	96 (11.2)	6.88 (5.7–8.2)
5–9	292 (28.0)	5.88 (5.1–6.7)	128 (15.0)	7.23 (6.1–8.5)
≥10	436 (41.9)	5.49 (4.8–6.2)	198 (23.2)	6.24 (5.3–7.3)
% Difference (≥10 vs. <5 years)		10.9%		10.2%
P_{cat}[e] (P_{trend})[f]		0.07 (0.01)		0.04 (0.24)
P adjusted for age at menopause		0.66 (0.28)		0.11 (0.37)
Previous hormone therapy use				
Never	824 (79.2)	5.60 (5.1–6.2)		
Previous	212 (20.4)	5.78 (5.1–6.5)		
P_{cat}[e]		0.38		
Duration of hormone therapy, y				
<1	63 (29.7)	5.79 (4.4–7.6)[c]	64 (8.2)	5.57 (4.7–6.6)
1–3	96 (45.3)	6.13 (4.8–7.9)[c]	271 (34.7)	6.43 (5.7–7.3)
≥4	45 (21.2)	7.13 (5.4–9.4)[c]	429 (55.0)	6.73 (6.0–7.6)
% Difference (≥4 vs. <1 years)		−18.8%[c]		−17.2%[g]
P_{cat}[e] (P_{trend})[f]		0.11 (0.01)[c]		0.02 (0.05)
Type of current hormone therapy used[d]				
Nonusers	1,021	5.81 (5.4–6.3)		
E alone			205	5.90 (5.3–6.5)
% Difference (E vs. nonuser)				1.6%
Estrogen+progestin (E+P)			415	6.66 (6.1–7.3)
% Difference (E+P vs. nonuser)				14.6%[g]
P_{cat} (E+P vs. E only)[e]				0.001

Abbreviations: E, estrogen; P, progesterone.

[a]Adjusted for center, age (continuous), time at blood donation (before 10 am, 10 am–1 pm, after 1 pm), fasting status (<3, 3–6, >6 hours), smoking (never, former, current, unknown), and parity (never, ever) where appropriate.

[b]The percentages do not add up to 100% because of missing values.

[c]In women who previously used hormone therapy.

[d]Subset of 620 women with known type of hormone therapy.

[e]Difference of adjusted geometric means among exposure categories (P_{cat}) was assessed by the type III test using F statistics obtained from linear regression models.

[f]Linear trends (P_{trend}) between prolactin levels and quantitative variables were tested by fitting linear regression models with continuous exposure variables.

[g]$P < 0.05$.

respect to breast cancer, in contrast, decreased risk of breast cancer in parous women correlates with age at first FTP (41). Although the pituitary gland is the major source of prolactin synthesis, extrapituitary prolactin produced locally in other tissues, including breast tissue, potentially acts as an autocrine–paracrine factor within the breast (42). However, less is understood about the role of extra pituitary prolactin during normal physiology and about mechanisms that control expression of prolactin at extra pituitary sites.

Because of a strong influential role of prolactin on the proliferation process of the lobuloalveolar epithelium of

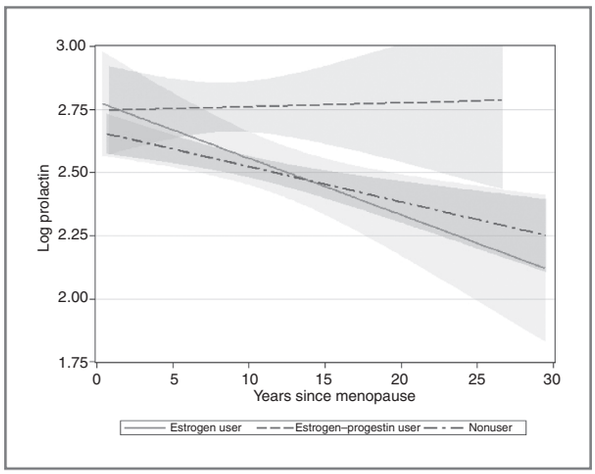

Figure 2. Log-transformed prolactin levels depending on years since menopause and type of hormone therapy used at the time of blood donation. Correlation coefficients between circulating prolactin levels and years since menopause are −0.04 (−0.20, 0.13), $P = 0.64$ for combined estrogen/progestin users; −0.20 (−0.35, −0.04), $P < 0.02$ for estrogen only users; and −0.17 (−0.23, −0.11), $P < 0.0001$ for postmenopausal non-hormone therapy users.

the breast during pregnancy and lactation (1), it would be plausible that breastfeeding influences prolactin levels beyond the effect of pregnancy. Indeed, a few small-scale studies with relatively young cohorts of women (median age 29 and 39 years) showed that prolactin was lowered by breastfeeding duration for the first child, with no substantial effect on feeding subsequent children (8, 9). However, the clear null result with respect to breastfeeding duration and prolactin levels in our study and another large prospective study (6) suggests that the association is diminished with time, if present at all. Nevertheless, it should be noted that the median total breastfeeding duration in our study population was 6.5 months and this relatively short duration and little variability across our population might result underestimation of the effect of breastfeeding on prolactin levels. Moreover, although prolactin is important in breast development and levels modestly increase during puberty (43), age at menarche has not been found to influence prolactin levels in current or in other studies (6, 11).

The lack of variation in prolactin levels in relation to physical activity or alcohol consumption suggests limited associations between prolactin and breast cancer–related lifestyle risk factors (7, 9, 44). However, the lower prolactin response related to smoking exposure confirmed in our study is believed to be the consequence of nicotine induced activation of dopamine secretion and its antiestrogenic action, which inhibit prolactin release among smokers (45, 46).

Obesity-related anthropometric parameters were inversely linearly associated with circulating prolactin in one large study (47) and among postmenopausal hormone therapy users in our study, but not in several other studies (9, 11, 44, 48). High serum prolactin has been also associated with favorable glucose metabolic profile and lower prevalence of diabetes (49, 50), which taken together could provide some evidence of obesity-related prolactin actions; however, substantially more data are needed to explore this relationship before conclusions can be drawn.

The strength of this study includes its extensive collection of information on reproductive and lifestyle factors and a large sample size, which allowed us to stratify the analyses by menopausal status and by postmenopausal hormone therapy use at the time of blood donation. In addition, our inter- and intra-assay correlations between prolactin measurements were low, showing a good validity of the data. The analyses presented here have some limitations. Our study has a cross-sectional design that cannot establish causality of the relationship seen between prolactin and studied factors. Prolactin has a strong circadian pattern, which could make the interpretation of single blood prolactin value rather complex. However, the observed circadian pattern together with other factors was controlled in our analyses. Because of no or limited information on family history of breast cancer, acute psychological stress and use of antidepressants/thyroid hormones in the EPIC study, we were not able to address the influence of these factors on prolactin levels.

In conclusion, our large-scale study shows that current hormone therapy use at the time of blood donation is associated with the higher circulating prolactin levels in postmenopausal women, with the effect confined to the women who were using combined estrogen–progestin therapy. Our study also confirmed previous findings indicating the permanent reduction of prolactin secretion after FTP. Moreover, the magnitude of this reduction depended on the number of FTPs. No major variation in prolactin levels was seen in relation to the other reproductive and lifestyle risk factors for breast cancer, with the exception of lower levels among postmenopausal women who were current smokers compared with never smokers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Horseman ND. Prolactin and mammary gland development. J Mamm Gland Biol Neopl 1999;4:79–88.
2. Ignacak A, Kasztelnik M, Sliwa T, Korbut RA, Rajda K, Guzik TJ. Prolactin–not only lactotrophin. A "new" view of the "old" hormone. J Physiol Pharmacol 2012;63:435–43.
3. Hankinson SE, Willett WC, Michaud DS, Manson JE, Colditz GA, Longcope C, et al. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. J Nat Cancer Inst 1999;91:629–34.
4. Tworoger SS, Eliassen AH, Zhang X, Qian J, Sluss PM, Rosner BA, et al. A 20-year prospective study of plasma prolactin as a risk marker of breast cancer development. Cancer Res 2013;73:4810–9.
5. Tikk K, Sookthai D, Johnson T, Rinaldi S, Romieu I, Tjønneland A, et al. Circulating prolactin and breast cancer risk among pre- and postmenopausal women in the EPIC cohort. Ann Oncol 2014;25:1422–8.
6. Eliassen AH, Tworoger SS, Hankinson SE. Reproductive factors and family history of breast cancer in relation to plasma prolactin levels in premenopausal and postmenopausal women. Int J Cancer 2007; 120:1536–41.
7. Tworoger S, Missmer S, Eliassen A, Barbieri R, Dowsett M, Hankinson S. Physical activity and inactivity in relation to sex hormone, prolactin, and insulin-like growth factor concentrations in premenopausal women. Cancer Causes Control 2007;18:743–52.
8. Hietala M, Olson H, Jernström H. Prolactin levels, breast-feeding and milk production in a cohort of young healthy women from high-risk breast cancer families: implications for breast cancer risk. Fam Cancer 2008;7:221–8.
9. Nagata C, Wada K, Nakamura K, Hayashi M, Takeda N, Yasuda K. Associations of body size and reproductive factors with circulating levels of sex hormones and prolactin in premenopausal Japanese women. Cancer Causes Control 2011;22:581–8.
10. Musey VC, Collins DC, Musey PI, Martino-Saltzman D, Preedy JRK. Long-term effect of a first pregnancy on the secretion of prolactin. N Engl J Med 1987;316:229–34.
11. Wang DY, De Stavola BL, Bulbrook RD, Allen DS, Kwa HG, Verstraeten AA, et al. The permanent effect of reproductive events on blood prolactin levels and its relation to breast cancer risk: a population study of postmenopausal women. Eur J Cancer Clin Oncol 1988; 24:1225–31.
12. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Pub Health Nutr 2002;5:1113–24.
13. Metka M, Holzer G, Raimann H, Heytmanek G, Hartmann B, Kurz C. The role of prolactin in the menopause. Maturitas 1994;20:151–4.
14. Schlegel W, Petersdorf LI, Junker R, Schulte H, Ebert C, Von Eckardstein A. The effects of six months of treatment with a low-dose of conjugated oestrogens in menopausal women. Clin Endocrinol 1999;51:643–51.
15. Stanosz SE, Zochowska E, Safranow K, Sieja K, Stanosz M. Influence of modified transdermal hormone replacement therapy on the concentrations of hormones, growth factors, and bone mineral density in women with osteopenia. Metabolism 2009; 58:1–7.
16. Balint-peric LA, Prelevic GM. Changes in prolactin levels with the menopause: the effects of estrogen/androgen and calcitonin treatment. Gynecol Endocrinol 1997;11:275–80.
17. Foth D, Römer T. Prolactin serum levels in postmenopausal women receiving long-term hormone replacement theraphy. Gynecol Obstet Invest 1997;44:124–6.
18. Abech DD, Moratelli HB, Leite CBF Sr, Oliveira MC. Effects of estrogen replacement therapy on pituitary size, prolactin and thyroid-stimulating hormone concentrations in menopausal women. Gynecol Endocrinol 2005;21:223–6.
19. Christiansen E, Veldhuis JD, Rogol AD, Stumpf P, Evans WS. Modulating actions of estradiol on gonadotropin-releasing hormone-stimulated prolactin secretion in postmenopausal individuals. Am J Obstet Gynecol 1987;157:320–5.
20. Halbreich U, Rojansky N, Palter S, Tworek H, Hissin P, Wang K. Estrogen augments serotonergic activity in postmenopausal women. Biol Psychiatry 1995;37:434–41.
21. Barlow DH, Beastall GH, Abdalla HI, Elias-Jones J, Lindsay R, Hart DM. Effect of long term hormone replacement on plasma prolactin concentrations in women after oophorectomy. Br Med J 1985; 290:589–91.
22. Sathyapalan T, Gonzalez S, Atkin SL. Effect of long-term, high-dose estrogen treatment on prolactin levels: a retrospective analysis. Climacteric 2009;12:427–30.
23. Wiegratz I, Kutschera E, Lee JH, Moore C, Mellinger U, Winkler UH, et al. Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. Contraception 2003;67: 25–32.
24. Lee PA, Xenakis T, Winer J, Matsenbaugh S. Puberty in girls: correlation of serum levels of gonadotropins, prolactin, androgens, estrogens, and progestins with physical changes. J Clin Endocrinol Metab 1976;43:775–84.
25. Tanner MJ, Hadlow NC, Wardrop R. Variation of female prolactin levels with menopausal status and phase of menstrual cycle. Aust NZ J Obstet Gynaecol 2011;51:321–4.

26. Xu B, Lipworth L, Wide L, Wuu J, Yu SZ, Lagiou P, et al. Maternal and gestational correlates of pregnancy prolactin and growth hormone in USA and China. Eur J Cancer Prev 2003;12:35–42.
27. Fink G. Oestrogen and progesterone interactions in the control of gonadotrophin and prolactin secretion. J Steroid Biochem 1988; 30:169–78.
28. Arbogast LA, Ben-Jonathan N. The preovulatory prolactin surge is prolonged by a progesterone-dependent dopaminergic mechanism. Endocrinol 1990;126:246–52.
29. Westberg L, Ho HP, Baghaei F, Nilsson S, Melke J, Rosmond R, et al. Polymorphisms in oestrogen and progesterone receptor genes: possible influence on prolactin levels in women. Clin Endocrinol 2004; 61:216–23.
30. Caufriez A, Leproult R, L'Hermite-Balériaux M, Moreno-Reyes R, Copinschi G. A potential role of endogenous progesterone in modulation of GH, prolactin and thyrotrophin secretion during normal menstrual cycle. Clin Endocrinol 2009;71:535–42.
31. Missmer SA, Spiegelman D, Bertone-Johnson ER, Barbieri RL, Pollak MN, Hankinson SE. Reproducibility of plasma steroid hormones, prolactin, and insulin-like growth factor levels among premenopausal women over a 2- to 3-year period. Cancer Epidemiol Biomarkers Prev 2006;15:972–8.
32. Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. Cancer Res 2006;66:2476–82.
33. Haisenleder DJ, Gala RR, Lawson DM. The effect of transient dopamine antagonism on thyrotropin-releasing hormone-induced prolactin release in female rats during the estrous cycle. Life Sci 1991;48: 1911–8.
34. Bottner M, Christoffel J, Jarry H, Wuttke W. Effects of long-term treatment with resveratrol and subcutaneous and oral estradiol administration on pituitary function in rats. J Endocrinol 2006;189:77–88.
35. Castelo-Branco C, Martinez de Osaba MJ, Fortuny A, Iglesias X, Gonzalez-Merlo J. Circulating hormone levels in menopausal women receiving different hormone replacement therapy regimens. A comparison. J Reprod Med 1995;40:556–60.
36. Haisenleder DJ, Moy JA, Gala RR, Lawson DM. The effect of transient dopamine antagonism on thyrotropin-releasing hormone-induced prolactin release in ovariectomized rats treated with estradiol and/or progesterone. Endocrinol 1986;119:1996–2003.
37. Ingram DM, Nottage EM, Roberts AN. Prolactin and breast cancer risk. Med J Aust 1990;153:469–73.
38. Dorgan JF, Reichman ME, Judd JT, Brown C, Longcope C, Schatzkin A, et al. Relationships of age and reproductive characteristics with plasma estrogens and androgens in premenopausal women. Cancer Epidemiol Biomarkers Prev 1995;4:381–6.
39. Hankinson SE, Colditz GA, Hunter J, Manson JE, Willett WC, Stampfer MJ, et al. Reproductive factors and family history of breast cancer in relation to plasma estrogen and prolactin levels in postmenopausal women in the Nurses' Health Study (United States). Cancer Causes Control 1995;6:217.
40. Bridges RS, Byrnes EM. Reproductive experience reduces circulating 17-estradiol and prolactin levels during proestrus and alters estrogen sensitivity in female rats. Endocrinol 2006;147:2575–82.
41. Warner E, Colditz G, Palmer J, Partridge A, Rosner B, Tamimi R. Reproductive factors and risk of premenopausal breast cancer by age at diagnosis: are there differences before and after age 40? Breast Cancer Res Treat 2013;142:165–75.
42. Muthuswamy SK. Autocrine prolactin: an emerging market for homegrown (prolactin) despite the imports. Genes Dev 2012;26:2253–8.
43. Aitkenhead H, Heales SJ. Establishment of paediatric age-related reference intervals for serum prolactin to aid in the diagnosis of neurometabolic conditions affecting dopamine metabolism. An Clin Biochem 2013;50:156–8.
44. Hankinson SE, Willett WC, Manson JE, Hunter DJ, Colditz GA, Stampfer MJ, et al. Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. J Nat Cancer Inst 1995;87: 1297–302.
45. Kapoor D, Jones TH. Smoking and hormones in health and endocrine disorders. Eur J Endocrinol 2005;152:491–9.
46. Glintborg D, Mumm H, Hougaard DM, Ravn P, Andersen M. Smoking is associated with increased adrenal responsiveness, decreased prolactin levels and a more adverse lipid profile in 650 white patients with polycystic ovary syndrome. Gynecol Endocrinol 2011;28:170–4.
47. Friedrich N, Rosskopf D, Brabant G, Völzke H, Nauck M, Wallaschofski H. Associations of anthropometric parameters with serum TSH, prolactin, IGF-I, and testosterone levels: results of the study of health in Pomerania (SHIP). Exp Clin Endocrinol Diabetes 2010;118:266–73.
48. Su X, Hankinson S, Clevenger C, Eliassen A, Tworoger S. Energy balance, early life body size, and plasma prolactin levels in postmenopausal women. Cancer Causes Control 2009;20:253–62.
49. Wang T, Lu J, Xu Y, Li M, Sun J, Zhang J, et al. Circulating prolactin associates with diabetes and impaired glucose regulation: a population-based study. Diabetes Care 2013;36:1974–80.
50. Balbach L, Wallaschofski H, Volzke H, Nauck M, Dorr M, Haring R. Serum prolactin concentrations as risk factor of metabolic syndrome or type 2 diabetes? BMC Endocr Disord 2013;13:12.